SPRAWOZDANIE TECHNICZNE: COLD JET - NOWOCZESNA TECHNIKA DO CZYSZCZENIA I ODKAŻANIA OBSZARÓW PRZETWÓRSTWA ŻYWNOŚCI, WYPOSAŻENIA, TUSZ I INNEJ ŻYWNOŚCI.

1.1 Tło

W nowoczesnych zakładach przetwórstwa spożywczego stosuje się różne metody czyszczenia i dezynfekcji powierzchni i urządzeń, które mają kontakt z żywnością podczas jej obróbki i przygotowywania. Często zakłady przetwórstwa spożywczego muszą najpierw oczyścić powierzchnie i urządzenia z poważnych zabrudzeń i zanieczyszczeń, i chociaż może to sprawić, że powierzchnie i urządzenia będą wyglądały na czyste, to nie dezynfekują ich. Czyszczenie i dezynfekcja w zakładach przetwórstwa spożywczego idą w parze, ponieważ widocznie czysta powierzchnia może nie być wolna od żywych mikroorganizmów, w szczególności tych, które mogą powodować zatrucia pokarmowe. Mikroorganizmy takie jak Salmonella, Listeria i E. coli są powszechnie znane jako przyczyny zatrucia pokarmowego, a choroby przenoszone przez żywność w wyniku spożywania żywności zanieczyszczonej tymi mikroorganizmami mogą być różne, od choroby i biegunki po poważne zagrożenia dla zdrowia osób, z możliwością długotrwałej choroby, a nawet śmierci. Dlatego też, oprócz uzyskania powierzchni, które są wyraźnie czyste, operacje przetwarzania żywności muszą dezynfekować (tj. odkażać) te powierzchnie i wyposażenie, które mają kontakt z żywnością.

Metody stosowane do czyszczenia obejmują użycie rozcieńczonych detergentów, wysokociśnieniowych węży wodnych, sprężonego powietrza, pary, ściereczek, materiałów ściernych (ręcznych lub mechanicznych), użycie środków chemicznych (na bazie chloru, innych firmowych środków dezynfekcyjnych), pary i innych systemów o wysokiej temperaturze/ciśnieniu. Stosowanie chemicznych środków dezynfekcyjnych może prowadzić do problemów, np. jeśli zastosowano niewłaściwe stężenie środka chemicznego lub jeśli nie ma wystarczającej ilości czasu na jego zadziałanie lub jeśli (w wyniku niewłaściwego użycia) na urządzeniach pozostały resztki środków czyszczących i dezynfekujących, które mogą zaszkodzić żywności lub niebezpieczeństwo użycia pary wodnej w środowisku fabrycznym, itp. Zdecydowano więc o zbadaniu zastosowania suchego lodu jako środka do czyszczenia i dezynfekcji urządzeń i obszarów przetwórstwa spożywczego.

1.2 Uzasadnienie i cele

Sposobem nanoszenia suchego lodu na powierzchnie przetwórstwa spożywczego był system Cold Jet, opatentowany system podawania granulatu suchego lodu w strumieniu powietrza i wydmuchiwanie ich z dyszy z dokładnie kontrolowaną prędkością. Celem było określenie, czy system Cold Jet może zarówno czyścić, jak i pomagać dezynfekować powierzchnie typowe dla procesów przetwórstwa spożywczego, np. płytki ceramiczne, stal nierdzewną, tworzywa sztuczne klasy spożywczej.

1.3 Podejście

Uzyskano przykłady rodzajów powierzchni stosowanych w przetwórstwie żywności i celowo zanieczyszczonych kulturami Salmonella enteritidis, Escherichia coli i Listeria monocytogenes. Następnie były one potraktowane granulatem suchego lodu przy użyciu systemu Cold Jet. Różne parametry systemu Cold Jet, takie jak natężenie przepływu powietrza, ciśnienie wydmuchiwania, natężenie przepływu granulatu suchego lodu itp. zostały zoptymalizowane w celu uzyskania jak najlepszego efektu usuwania tych mikroorganizmów.

System Cold Jet został również przetestowany w zakładzie produkującym paszę dla zwierząt w celu określenia jego przydatności do stosowania w środowisku spożywczym oraz jego przydatność do odkażania tuszek drobiowych i porcji mięsa poddanych ocenie.

1.4 Uzyskane wyniki

System Cold Jet do czyszczenia suchym lodem okazał się skuteczny w czyszczeniu i dezynfekcji różnych rodzajów powierzchni, w tym stali nierdzewnej, płytek ceramicznych, tworzyw sztucznych klasy spożywczej oraz powłok z tworzyw sztucznych.

System Cold Jet skutecznie pomaga w odkażaniu powierzchni z bakterii Salmonella, Escherichia coli i Listeria w taki sposób, że mikroorganizmy te nie były wykrywalne przy użyciu konwencjonalnych metod mikrobiologicznych. Docelowo zmniejszono populację bakterii o współczynnik 10.000 lub więcej i wykazano doświadczalnie, że było to spowodowane połączeniem granulatu suchego lodu i sprężonego powietrza.

Określono parametry optymalnego zastosowania systemu Cold Jet na powierzchniach, w tym średnicę granulatu suchego lodu, natężenie przepływu granulatu, ciśnienie wydmuchiwania, natężenie przepływu powietrza, czas czyszaczenia (czas działania suchego lodu na powierzchnię).

Stwierdzono, że niektóre powierzchnie są zbyt kruche do zastosowania systemu Cold Jet, np. niektóre gatunki tworzyw sztucznych zmieniły kształt i wygląd. Większość badanych tworzyw spożywczych wytrzymała jednak tę obróbkę.

System Cold Jet okazał się skuteczny w czyszczeniu i dezynfekcji zakładów przetwórczych po wstępnym oczyszczeniu go w celu usunięcia większych zanieczyszczeń. Zastosowanie urządzenia Cold Jet bezpośrednio na mięsie i drobiu zmniejszyło liczbę występujących w nim mikroorganizmów, ale spowodowało niedopuszczalne uszkodzenie tkanek w wyniku działania granulatu suchego lodu i sprężonego powietrza.

1.5 Wnioski

System Cold Jet okazał się skuteczny w czyszczeniu i dezynfekcji różnych powierzchni przeznaczonych do kontaktu z żywnością. Ma wyraźną przewagę nad konwencjonalnymi technikami czyszczenia i dezynfekcji, ponieważ po obróbce nie pozostały żadne pozostałości chemiczne, a odpady chemiczne nie byłyby poddawane obróbce i usuwaniu. Na obecnym etapie system Cold Jet może być wykorzystywany do dezynfekcji operacji przetwarzania żywności, najlepiej po początkowym usunięciu dużych resztek.

Kolejnym etapem byłoby zbadanie integracji systemu Cold Jet z liniami przetwórstwa spożywczego, w których przeprowadzane są pewne operacje, w których obecnie nie jest możliwe wdrożenie polityki czyszczenia i dezynfekcji na bieżąco, np. eliminacja drobiu, patroszenie zwierząt / drobiu, ale tam, gdzie istnieje wysokie ryzyko zanieczyszczenia żywności. System Cold Jet może być wbudowany jako stała instalacja, umożliwiająca ciągłe czyszczenie i dezynfekcję, a także częstsze czyszczenie i dezynfekcję linii do przetwarzania żywności.

Zapraszamy do zapoznania się poniżej z kompletnym raportem w wersji angielskiej: SPRAWOZDANIE TECHNICZNE: COLD JET - NOWOCZESNA TECHNIKA DO CZYSZCZENIA I ODKAŻANIA OBSZARÓW PRZETWÓRSTWA ŻYWNOŚCI, WYPOSAŻENIA, TUSZ I INNEJ ŻYWNOŚCI.

CONTRACTORS REPORT TO THE FOOD STANDARDS AGENCY

FINAL TECHNICAL REPORT: COLD JET - A NOVEL TECHNIQUE FOR CLEANING AND DECONTAMINATING FOOD PROCESSING AREAS, EQUIPMENT, CARCASSES AND FOODS.

Title: Cold Jet – A novel technique for cleaning and decontaminating food processing areas, equipment, carcasses and foods.

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Start Date:	01/02/1999
Finish Date:	31/01/2002
Report Date:	19/09/2004

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1. Executive Summary:

1.1 Background

Modern food processing operations use a variety of methods for cleaning and disinfecting surfaces and equipment which come into contact with foods during handling and preparation. Often food processing factories have to conduct an initial clean up of gross dirt and debris, and although this may leave surfaces and equipment looking clean, this does not disinfect those surfaces. Cleaning and disinfection in food processing operations go hand in hand as a visibly clean surface may not be free from viable microorganisms, in particular those microorganisms that can cause food poisoning. Microorganisms such as *Salmonella*, *Listeria*, and *E. coli* are well known to the public as causes of food poisoning, and foodborne illness due to consumption of foods contaminated with these microorganisms can vary from sickness and diarrhoea through to serious health risks to individuals, with the possibility of long term illness, or even death. Thus as well as obtaining surfaces that are visibly clean, food processing operations must disinfect (i.e. sanitise) those surfaces and equipment that come into contact with foods.

Methods used to clean include using diluted detergents, high pressure water hoses, compressed air, steam, cloths, abrasives (manual or mechanical), whilst methods used to disinfect include the use of chemicals (e.g. hypochlorites and other types of chlorine-based chemicals, other proprietary disinfectants), steam and other high temperature/pressure systems. The use of chemical disinfectants can lead to problems, e.g. if the wrong concentration of chemical is used, or if insufficient time is allowed for the chemical to work, or if (through incorrect usage) residues of cleaning and disinfecting chemicals are left on equipment where they can then taint the food, or the danger of using steam in a factory environment, etc. Thus it was decided to investigate the use of blasting dry ice as a means of both cleaning and disinfecting food processing equipment and areas.

1.2 Rationale and Objectives

The means of applying dry ice to food processing surfaces was the Cold Jet system, a patented system for dispensing pellets of dry ice by mixing them with compressed air and blasting them from a nozzle at carefully controlled rates. The objectives were to determine whether the Cold Jet system could both clean and disinfect the types of surfaces typically found in food processing operations, e.g. ceramic tiles, stainless steel, food grade plastics.

1.3 Approach

Examples of the types of surfaces used in food processing operations were obtained and deliberately contaminated with cultures of *Salmonella enteritidis*, *Escherichia coli*, and *Listeria monocytogenes*. They were then blasted with dry ice pellets using the Cold Jet system. Various parameters of the Cold Jet system such as air flow rate, blast pressure, dry ice pellet flow rate, etc., were optimised to give the greatest possible ‘kill’ effect on these microorganisms.

The Cold Jet system was also tested in an animal feed production facility in order to determine its suitability for use in a food environment (contaminated animal feeds are

a significant source of food poisoning microorganisms in foods; Crump *et al* 2002), and its suitability for decontaminating poultry carcasses and meat portions evaluated.

1.4 Outcome / Key Results Obtained

The Cold Jet system for applying dry ice pellets onto surfaces has been shown to be effective in cleaning and disinfecting various types of surfaces, including stainless steel, ceramic tiles, and food grade plastics and plastic surface coatings.

The Cold Jet system effectively decontaminated surfaces of *Salmonella enteritidis*, *Escherichia coli*, and *Listeria monocytogenes* such that these microorganisms were not detectable using conventional microbiological methods after a defined Cold Jet treatment. Decreases in target bacterial populations by a factor of 10,000 or more were obtained, and that this was due to the combined application of dry ice pellets and compressed air was shown experimentally.

The parameters for optimum application of the Cold Jet system on surfaces were determined, including diameter of dry ice pellets, pellet flow rate, blast pressure, air flow rate, blasting time (time of application of dry ice pellets to surfaces).

Some surfaces were found to be either too brittle or too fragile for the application of the Cold Jet system, e.g. certain grades of plastics changed shape and appearance, or cracked or peeled, but the majority of food grade plastics tested withstood the treatment.

Cold Jet was found to be effective in cleaning and disinfecting processing plant after it had received an initial clean to remove gross debris. When Cold Jet was used directly on meat and poultry it did decrease the numbers of microorganisms present, but caused unacceptable tissue damage due to the action of the dry ice pellets and compressed air.

1.5 Conclusions and Possible Future Work

The Cold Jet system has been shown to be effective in cleaning and disinfecting various food grade surfaces. It has a distinct advantage over conventional cleaning and disinfection techniques in that there are no chemical residues left behind after treatment, and no chemical wastes to treat and dispose of. As it stands, the Cold Jet system could be used now to disinfect food processing operations, preferably after an initial removal of gross debris.

The next stage would be to investigate the integration of the Cold Jet system into food processing lines, where certain operations are conducted where a clean and disinfect-as-you-go policy is not currently feasible, e.g. poultry defeathering, animal/poultry evisceration, but where there is a high risk of contamination of foods. The Cold Jet system could be built-in as a fixed installation to allow continuous cleaning and disinfection, or else more frequent cleaning and disinfection, of the food processing line.

2. Glossary:

BPW: Buffered Peptone Water

BPWGL: Buffered Peptone Water Glucose Lysine

CCP's: Critical Control Points

CFM: Cubic Feet per Minute

FSA: Food Standards Agency

HACCP: Hazard Analysis Critical Control Point

MRD: Maximum Recovery Diluent

NA: Nutrient Agar

NB: Nutrient Broth

PSI: Pounds per Square Inch

TSA: Tryptone Soya Agar

TSB: Tryptone Soya Broth

TVC: Total Viable Count

UV: Ultra Violet

XLD: Xylose-Lysine-Desoxycholate agar

3. Aim and Objectives of the Investigation:

In food processing operations it is of critical importance that these are conducted in hygienically clean conditions, i.e. all surfaces and equipment which the food is in contact with are both clean and sanitised, and that all food handling personnel are aware of the importance of good personal hygiene and of the need to maintain adequate hygiene with food processing plant and equipment. In particular the need to minimise the risk of contamination with (and growth of) pathogenic microorganisms, which if ingested by the final consumer of the food may lead to food poisoning, must be addressed.

The inadvertent transfer of bacteria from the hide and gut to the surfaces of animal carcasses during slaughter and dressing is inevitable with current slaughterhouse technology. It is considered that the gut and hide will contain some level of potential pathogens which are of public health concern, and it is the aim of modern slaughtering and dressing operations to minimise cross contamination from these to carcass meat to the lowest possible level (Biss & Hathaway, 1998).

Microorganisms can adhere to a variety of natural and man made surfaces commonly found in food processing factories, and once attached these microorganisms can multiply and form biofilms which are difficult to remove and can act as a source of contamination for food products (Biss & Hathaway, 1998).

There are a variety of methods that can be used to clean and disinfect food processing surfaces and equipment including:

- brushing/rinsing with water followed by use of chemical cleaners and disinfectants, e.g. hypochlorites, detergent sanitisers;
- high pressure water hoses (with or without detergent sanitisers);
- steam;
- Ultra Violet (UV) irradiation, primarily used to disinfect water systems and in the treatment of factory effluent;

- ozone gas has been used to sterilise discharge water from fish processing factories and to sterilise raw fish materials.

There are numerous disadvantages with each of these methods, e.g. chemical disinfectants (hypochlorites, detergent sanitisers) may not be used at the correct concentration or left for the appropriate contact time; high pressure water hoses cause aerosols and can spread microbiological contamination; steam can be dangerous and also cause aerosols; UV irradiation has limited applications in food processing; gamma irradiation is effective but not popular with consumers; ozone gas is hazardous.

The control of microbiological hazards in food processing is a complex one, involving many aspects including; hygiene training of food processing personnel; food processing plant sanitation; quality and safety control procedures, e.g. microbiological analyses on incoming raw materials and final product(s); physical and chemical factors, e.g. process and storage temperatures, pH, salt concentration, etc.; shelf life of product; projected end use of food product. Today all of these complex factors are generally assessed, monitored and controlled by using a risk assessment based approach - Hazard Analysis Critical Control Point (HACCP). This is done by conducting a systematic assessment of the food production process and drawing up a HACCP plan covering all aspects of the food product, from supply and sourcing of raw materials through to storage and use of end product by the consumer. A key part of any HACCP plan involves monitoring of Critical Control Points (CCP's) to ensure that the process is under control. In a food processing operation, it is vital that all surfaces and equipment in a food processing plant that come into direct contact with the foods being prepared are properly cleaned and disinfected, thus it is important that suitable monitoring procedures are put in place to ensure that all surfaces are hygienically clean.

The Cold Jet system cleans surfaces by gently spraying them with pellets of dry ice (solid carbon dioxide - CO_2) at a temperature of -78.5°C. On impact, the dry ice pellets sublimate directly from solid to gas, and the Cold Jet system uses this combination of kinetic energy and thermal shock to cause cracking of the analyte on

the surface. The thermal shock causes the coating or contaminant material (including microorganisms and food residues on surfaces being cleaned) to thermally contract before any thermal contraction of the parent surface or substrate underneath the contaminant, thus breaking the bond between the coating or contaminant and the parent surface. The compressed air used to deliver the pellets to the surface then lifts the coating or contaminant from the parent surface and it is carried away in the compressed air stream. It can then either be collected for disposal or vented to the atmosphere.

The Cold Jet system was originally developed for use in the aerospace and nuclear industries where cleaning of sensitive components, that were often difficult to access, was required. The Cold Jet system allowed cleaning or stripping of surfaces without causing damage to the underlying material. Thus for example Cold Jet is used to remove paint from aircraft without causing damage to the underlying aircraft structure. Since then the use of Cold Jet has spread to include cleaning of extrusion moulds for the rubber and plastic industries, cleaning stonework, etc. A comprehensive history of Cold Jet can be found at the company's website at www.coldjet.com.

The main aim and objective of this project was to evaluate the Cold Jet system for the cleaning and disinfection of food processing areas, food processing equipment, and foodstuffs. The Cold Jet system was evaluated for its ability to both clean and disinfect a wide range of the materials found in food processing factories, including stainless steel, ceramic tiles, food grade plastics, and plastic surface coatings.

4. Experimental Procedures:

4.1 Microbiological Media

Nutrient Broth (NB), Nutrient Agar (NA), Tryptone Soya Broth (TSB), Tryptone Soya Agar (TSA), Buffered Peptone Water (BPW), Maximum Recovery Diluent (MRD) and Xylose-Lysine-Desoxycholate (XLD) agar were obtained from Oxoid (Oxoid Limited, Basingstoke, Hants).

4.2 Biochemicals & Bacterial Cultures

L-Lysine monohydrochloride was obtained from Sigma (Sigma-Aldrich Chemical Co., Poole, Dorset). D-Glucose was obtained from Merck (Merck Limited, Magna Park, Lutterworth, Leics.).

The following stock cultures were used; *Salmonella enteritidis* NCTC 12694, *Escherichia coli* NCIMB 11595, and *Listeria monocytogenes* NCTC 11994. Purity of stock cultures was determined by streak plating onto nutrient agar for *S. enteritidis* and *E. coli*, and tryptone soya agar for *L. monocytogenes*. Stock cultures of *S. enteritidis* and *E. coli* were maintained on nutrient agar slants, and stock cultures of *L. monocytogenes* were maintained on tryptone soya agar slants. All stock cultures were maintained at room temperature.

4.3 Test surfaces

Various surfaces as found and used in food processing factories were obtained from various companies for use in the Cold Jet trials. Details of these are given in Table 4.1.

Surfaces were prepared for use by soaking and washing in hot water and commercial liquid detergent (used at a concentration of 0.2%) for 5 minutes, rinsing with hot and cold water, and allowed to air dry overnight at room temperature. Surfaces were then individually wrapped in autoclave bags, and sterilised by autoclaving at 121°C for 15 minutes.

Table 4.1. Surfaces used in Cold Jet trials

Surface No.	Material	Supplier
1	Chopping Board (used, polypropylene)	Alex Ross Ltd.
2	Chopping Board (used, polypropylene)	Alex Ross Ltd.
3	Stainless steel bracket	Alex Ross Ltd.
4	Conveyor Belt – plastic	Alex Ross Ltd.
5	Stainless Steel	Alex Ross Ltd.
6	Polypropylene	ABG Rubber & Plastics Ltd.
7	PE500	ABG Rubber & Plastics Ltd.
8	Acetal	ABG Rubber & Plastics Ltd.
9	PETP Ertalyte	ABG Rubber & Plastics Ltd.
10	Vivak	ABG Rubber & Plastics Ltd.
11	1. Stainless steel AISI 304, glass-blasted surface treatment	Norfo A/S
12	2. Stainless steel, no surface treatment	Norfo A/S
13	3. Stainless steel, polished surface	Norfo A/S
14	4. White Nylon plate, used for cutting plates, etc.	Norfo A/S
15	5. Sample of transport belt used in Portion Cutter	Norfo A/S
16	6. Circular Saw blade	Norfo A/S
17	7. Band Saw	Norfo A/S
18	Sterisept	Liquid Plastics Ltd.
19	Steridex	Liquid Plastics Ltd.
20	Steridex on brushed aluminium	Liquid Plastics Ltd.
21	Steridex on MDF	Liquid Plastics Ltd.
22	Sterisheen on MDF	Liquid Plastics Ltd.
23	Sterisept – Reemal lite	Liquid Plastics Ltd.
24	Sterisept & Sterisheen on tiles	Liquid Plastics Ltd.
25	Sterisept & Sterisheen on tiles. Both surface treatments worn.	Liquid Plastics Ltd.
26	Plain white ceramic tiles	B & Q
27	Plain red quarry tiles	B & Q

4.4 Cold Jet System

A Cold Jet RDS-500-Cub was provided by Cold Jet (Europe) Ltd., Wisbech, Cambs.

Air compressors capable of running at between 260 and 400 Cubic Feet per Minute (CFM) were either loaned or hired from Coates Rentair for use during experimental trials.

4.5 Carbon Dioxide (CO_2) Pellets (Dry Ice)

Solid carbon dioxide (CO_2) pellets (dry ice) were obtained from Hydro Gas & Chemicals (Drikold Pellets, Hydro Gas & Chemicals Limited, Immingham, Lincs).

4.6 Preparation of bacterial cultures (Dip Cultures) for surface seeding experiments

4.6.1 *S. enteritidis* (NCTC 12694)

Cultures were prepared by inoculating 5ml BPW with a loopful of stock culture of *S. enteritidis*, incubating overnight at 37°C, then transferring this 5ml culture into 100ml BPW and incubating overnight at 37°C. 10mls of this culture was then added to 990ml MRD, giving an overall dilution factor of 10^{-2}, thus assuming an overnight culture contained 10^8 cells.ml^{-1} then this would give a liquid culture for dipping test surfaces that contained 10^6 cells.ml^{-1}. (This was confirmed for each experimental trial - data not shown. TVC's per ml for overnight cultures ranged between 5.6×10^8 to 8.2×10^8).

4.6.2 *E. coli* 11595

Cultures were prepared by inoculating 5ml NB with a loopful of stock culture of *E. coli*, incubating overnight at 37°C, then transferring this 5ml culture into 100ml NB and incubating overnight at 37°C. 10mls of this culture was then added to 990ml MRD, giving an overall dilution factor of 10^{-2}, thus assuming an overnight culture contained 10^8 cells.ml^{-1} then this would give a liquid culture for dipping test surfaces that contained 10^6 cells.ml^{-1}. (This was confirmed for each experimental trial - data not shown. TVC's per ml for overnight cultures ranged between 1.4×10^8 to 3.5×10^8).

4.6.3 *L. monocytogenes* 11994

Cultures were prepared by inoculating 5ml TSB with a loopful of stock culture of *L. monocytogenes*, incubating overnight at 37°C, then transferring this 5ml culture into 100ml TSB and incubating overnight at 37°C. 10mls of this culture was then added to 990ml MRD, giving an overall dilution factor of 10^{-2}, thus assuming an overnight culture contained 10^8 cells.ml^{-1} then this would give a liquid culture for dipping test surfaces that contained 10^6 cells.ml^{-1}. (This was confirmed for each experimental trial - data not shown. TVC's per ml for overnight cultures ranged between 1.4×10^8 to 3.4×10^8).

4.7 Seeding of test surfaces for Cold Jet trials

Dip cultures prepared as in 4.6 were used to inoculate test surfaces given in Table 4.1. Whole surfaces were immersed for 5 minutes in the dip cultures without agitation, then removed and allowed to drain by standing on edge for 3 minutes. Whole surfaces were used as the surfaces supplied listed in Table 4.1 were supplied by various companies and were not to a standard size or shape. Surfaces were then either swabbed (4.11) for Total Viable Count (TVC) determination (4.12), or treated with Cold Jet (4.8), or treated with air-blasting (4.9), or treated with CO_2 pellets (4.10). Surfaces were moist prior to either direct swabbing, or Cold Jet treatment and swabbing.

4.8 Treatment of test surfaces with Cold Jet

Test surfaces seeded as in 4.7 were treated with Cold Jet within the following parameter ranges (for details of actual parameters used, see Results & Discussion):

Cold Jet Outlet Blast Pressure	30 to 50 psi.;
Cold Jet Pellet Feeder Rate	30 to 60 (arbitrary scale on Cold Jet);
CO_2 pellets	3mm diameter.

Test surfaces were treated for various times (0 to 30 seconds), and immediately after treatment the surfaces were swabbed for determination of TVC (4.11). Individual test surfaces were treated in duplicate trials.

4.9 Treatment of surfaces with air-blasting only

Test surfaces seeded as in 4.7 were treated by blasting compressed air with Cold Jet within the following parameter ranges (for details of actual parameters used, see Results & Discussion):

Cold Jet Outlet Blast Pressure	30 to 50 psi.;
Cold Jet Pellet Feeder Rate	30 to 60;
CO_2 pellets	NOT USED.

Test surfaces were treated for various times by air blasting through the Cold Jet without any CO_2 pellets (0 to 30 seconds), and immediately after treatment the surfaces were swabbed for determination of TVC (4.11). Individual test surfaces were treated in duplicate trials.

4.10 Treatment of surfaces with carbon dioxide (CO_2) pellets (dry ice)

Test surfaces seeded as in 4.7 were treated by covering the surfaces with dry ice pellets (3mm diameter) for set periods (0 to 30 seconds). A double layer of dry ice pellets was used in order to ensure as even a coverage of the surfaces as possible. Dry ice pellets were removed by gently tipping the surfaces, and the surfaces were then immediately swabbed for determination of TVC (4.11).

4.11 Swabbing of surfaces

Surfaces that had been treated as in 4.8 - 4.10 above were swabbed to determine TVC. Sterile cotton wool swabs (Sterilin, Stone, Staffs.) were moistened using sterile MRD, then used to swab either the entire surface, or else a set area of the treated surface. Whole surfaces that were swabbed include tiles and plastics samples (Surface nos. 6 - 10, 18 - 25, 26 & 27; Table 4.1), whilst all other surfaces were swabbed over a set area. Swabs were then placed in 10ml MRD, and decimal dilutions prepared in MRD. The appropriate decimal dilutions were plated as in 4.12 to determine TVC.

4.12 Determination of Total Viable Count (TVC)

Total viable counts (TVC's) were determined by plating using the Modified Miles Misra technique (Bousefield *et al*, 1973). Decimal dilutions prepared in MRD were plated on NA (*S. enteritidis* or *E. coli*), or on TSA (*L. monocytogenes*), incubated at 37°C for 20 - 24 hours, counted and the TVC determined. All dilutions were plated in duplicate.

4.13 Detection and identification of *Salmonella* species

The method used was based on the AOAC Automated Conductance Method (AOAC, 1998). Swabs for *Salmonella* spp. were incubated in Buffered Peptone Water Glucose Lysine (BPWGL) at 37°C for 18 hours, then 100μL volumes of pre-enrichment were inoculated into Salmonella Medium 1 and Salmonella Medium 2 selective media (AOAC, 1998). SM1 and SM2 were incubated in a Malthus Microbiological Growth Analyser (IDG Limited, Bury, Lancs.) at 37°C for 24 hours. Suspect positive *Salmonella* spp. were plated on Oxoid Xylose-Lysine-Desoxycholate (XLD) agar, incubated at 37°C for 24 hours. Presumptive *Salmonella* spp., on XLD were confirmed using Microgen Microscreen *Salmonella* latex agglutination kit (Microgen Bioproducts Limited, Camberley, Surrey) and API20E (bioMerieux UK Limited, Basingstoke, Hants.).

4.14 Determination of suitability of Cold Jet for use in food processing premises

The Cold Jet system was evaluated at a fishmeal manufacturing facility. It is known that fishmeal can contain *Salmonella* species and other pathogenic Enterobacteriaceae, and that these microorganisms can subsequently be present in cattle and poultry that have had fishmeal in their diet. Therefore the Cold Jet system was evaluated in conjunction with the usual weekly deep-clean and disinfection procedures at this facility. The Cold Jet system was tested under the following conditions:

Cold Jet Outlet Blast Pressure	10 to 50 psi.;
Cold Jet Pellet Feeder Rate	10 to 30;

CO_2 pellets 3mm diameter.

Due to the heavy build up of debris in the fishmeal plant (after initial cleaning trials at the lower ranges of outlet blast pressure and pellet feeder rate above) the Cold Jet system was used at an outlet blast pressure of 50 psi and a pellet feeder rate of 30 in order to provide maximum cleaning effect.

Swabs (prepared as in 4.11) were taken of surfaces before and after Cold Jet treatment. Surfaces tested are given in Table 4.2. After sampling, swabs were placed in a cool box with ice packs, and transported to the laboratory and plated onto NA within 4 hours of sampling. All plates were incubated at 37°C for 20 - 24 hours, counted and the TVC determined. All dilutions were plated in duplicate.

Surfaces were also swabbed for *Salmonella* species analysis, after which the swabs were placed in 10ml BPWGL and placed in a cool box with ice packs, and transported to the laboratory. On receipt at the laboratory, the swabs were tested for the presence of *Salmonella* species as in 4.13.

Table 4.2 Surfaces treated with Cold Jet in a fishmeal processing plant

Swab Number	Location
1	Bottom Screw
2	Bottom Hatch
3	Rear of Main Access Hatch
4	RHS of Main Access Hatch
5	Cross Member up from Hatch
6	Screw next to Main Access Hatch
7	Rear side groove
8	Rear Screw
9	Underside rear cross member
10	Rear sampling / dumping point
11	RHS of Main Access Hatch
12	Rear Panel opposite Access Hatch
13	Screw next to Main Access Hatch
14	Bracket and Small Ledge above Screw adjacent to Main Access Hatch
15	Rear Slope above Screw
16	Central Drive Shaft of Screw
17	Rear side Groove/Slot above Screw
18	Central Drive Shaft
19	Screw towards rear of Shaft
20	Top of rear cross member

4.15 Determination of suitability of Cold Jet for use in decontamination of meat and poultry portions

Meat portions (silverside joints) and whole fresh chickens were purchased from local supermarkets. Large 3.5kg silverside joints were portioned into approximately 500g pieces prior to seeding with test bacteria. Whole fresh chickens (approximately 2.5kg each) were used for seeding experiments. In order to assess the efficiency of treatment with Cold Jet in removing foodborne pathogens, cultures of *S. enteritidis*, *E. coli* and *L. monocytogenes* were prepared as in Section 4.6. However, instead of dipping meat and poultry portions in liquid cultures, the diluted overnight cultures were sprayed onto the surfaces of the meat and poultry portions using a plant sprayer. Meat or poultry surfaces were sprayed prior to treatment with Cold Jet and allowed to stand for 10 minutes at room temperature prior to Cold Jet treatment.

For determination of TVC's, portions were swabbed as in 4.11 and TVC's determined as in 4.12. TVC's are the mean of 5 samples (5 separate meat/chicken portions). Areas swabbed were $5cm^2$.

Where there was damage to meat and poultry portions after treatment with Cold Jet, portions of meat or poultry were collected aseptically, and 25g of meat or poultry diluted 1 in 10 in MRD and plated onto NA (*S. enteritidis* or *E. coli*) or TSA plates (*L. monocytogenes*), incubated @ 37°C for 20 - 24h, and the TVC's determined.

It was not possible to sterilise meat and poultry portions prior to these trials, therefore there would be the normal microflora present on these samples (as well as the cultures added by spraying). The aim was to measure the overall effect of Cold Jet on reducing the total microbial population on meat and poultry portions.

4.16 Airborne contamination

Airborne contamination around the areas where the Cold Jet system was used was assessed using scatter plates, and also a Merck MAS 100 Air Sampler (Merck Limited, Magna Park, Lutterworth, Leics.). Media used included NA, TSA and XLD agars, plates were incubated @ 37°C for 20 - 24h following exposure, and then counted. Scatter plates were placed at between 1.5 and 3 metres from the point at which Cold Jet was being applied.

5. Results:

5.1 Effect of Cold Jet on various food-grade surfaces

The results for using the Cold Jet system on various types of surfaces found in food processing premises and with which food may come into contact are given in Table 5.1 with example data shown in Figures 5.1 & 5.2 (Raw data are supplied in Appendix A). All surfaces were seeded with *S. enteritidis*.

Table 5.1 Results of Cold Jet treatment on various surfaces

Surface No.	Material	TVC Pre Cold Jet	TVC Post Cold Jet
1	Chopping Board (used, polypropylene)	5.0×10^5	1.3×10^4
2	Chopping Board (used, polypropylene)	6.4×10^5	3.8×10^3
3	Stainless steel bracket	3.7×10^6	5.8×10^2
4	Conveyor Belt – plastic	2.3×10^6	1.2×10^5
5	Stainless Steel	8.4×10^6	3.0×10^2
6	Polypropylene	4.6×10^5	2.7×10^3
7	PE500	3.0×10^5	2.0×10^4
8	Acetal	3.2×10^5	4.0×10^2
9	PETP Ertalyte	3.1×10^5	1.0×10^3
10	Vivak	7.4×10^5	8.0×10^2
11	1. Stainless steel AISI 304, glass-blasted surface treatment	2.4×10^6	7.0×10^2
12	2. Stainless steel, no surface treatment	1.3×10^5	0
13	3. Stainless steel, polished surface	4.7×10^6	3.0×10^2
14	4. White Nylon plate, used for cutting plates, etc.	6.5×10^6	3.7×10^3
15	5. Sample of transport belt used in Portion Cutter	8.2×10^6	2.4×10^5
16	6. Circular Saw blade	6.7×10^6	2.7×10^2
17	7. Band Saw	4.3×10^6	4.4×10^2
18	Sterisept	Destroyed	Destroyed
19	Steridex	9.0×10^5	1.1×10^2
20	Steridex on brushed aluminium	8.7×10^5	0
21	Steridex on MDF	1.2×10^6	2.2×10^3
22	Sterisheen on MDF	1.9×10^6	6.3×10^3
23	Sterisept – Reemal lite	Destroyed	Destroyed
24	Sterisept & Sterisheen on tiles	Destroyed	Destroyed
25	Sterisept & Sterisheen on tiles. Both surface treatments worn.	Destroyed	Destroyed

Figure 5.1 TVC of various surfaces pre and post Cold Jet treatment

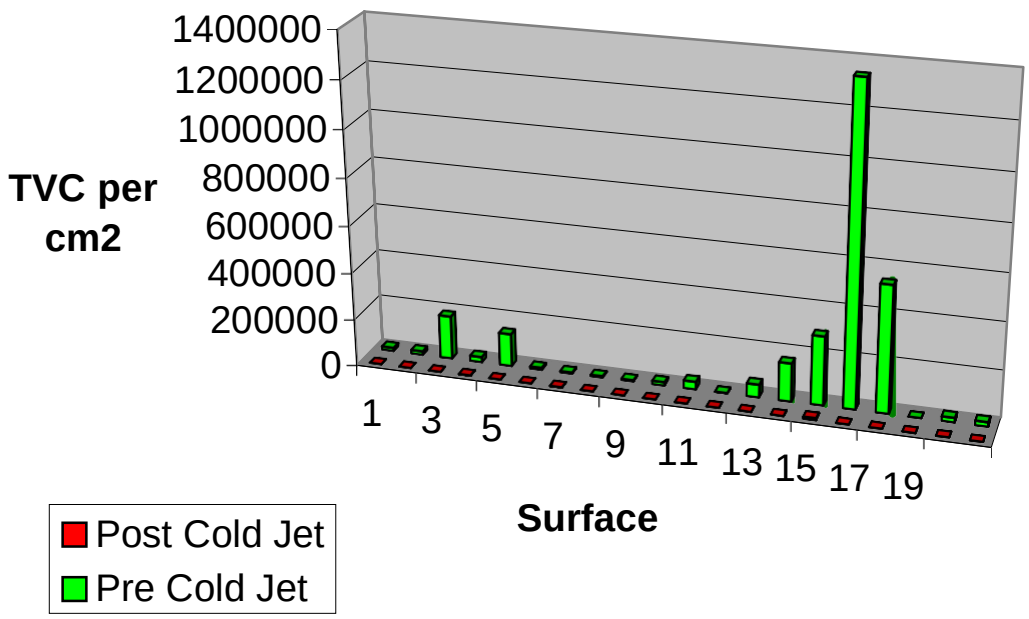
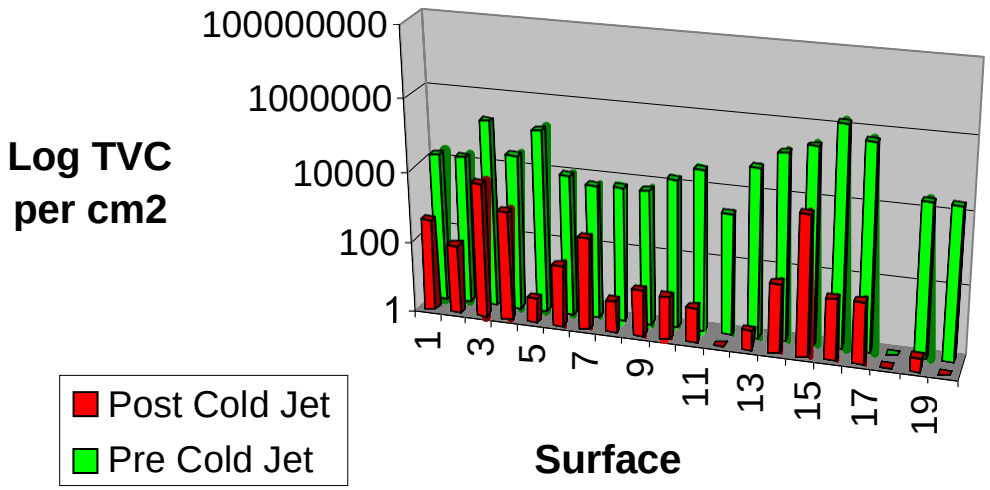


Figure 5.2 TVC of various surfaces pre and post Cold Jet treatment



Surfaces were subjected to 15 seconds of Cold Jet application with 3mm diameter CO_2 pellets, and pellet feeder rates of between 30 and 60. The initial outlet blast pressure was set at 50 psi, but this proved too fierce for some of the surfaces, therefore the outlet blast pressure was reduced to 30 psi. It is evident from Table 5.1 (and selected examples in Figures 5.1 & 5.2) that a reduction in TVC's was achieved with all surfaces, ranging from 1.5 to 5.0 log reductions in TVC's. For example the used polypropylene chopping board (surface no. 1) showed a 1.5 log reduction in TVC, whilst plain stainless steel (surface no. 12) showed a 5.0 log reduction in TVC after Cold Jet application.

Some of the surfaces tested proved too fragile for Cold Jet treatment, e.g. Sterisept (surface no. 18) was destroyed, and 'Vivak' (a propriety plastic surface; surface no. 10) was warped after treatment.

5.2 Effect of Cold Jet treatment on plain white ceramic tiles seeded with *S. enteritidis*

The effect of different Cold Jet application times was determined by treating identical plain white ceramic tiles (surface 26, Table 4.1) seeded with *S. enteritidis*. These tiles were chosen as they are typical of the sort found on walls and other surfaces in a wide variety of food processing operations, both large and small. Tiles (15cm^2) were subjected to blasting at either 0, 5, 10, 15, 20 or 30 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 30. The entire surface of the coated side of the tiles (the side that would face outwards from a surface to which the tile was fixed) was swabbed either before or after Cold Jet treatment. The results are shown in Figures 5.3 - 5.6 (Raw data are supplied in Appendix B) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is a 2.0 log reduction in the TVC after 5 seconds, and with 30 seconds of Cold Jet application there is a 3.0 - 4.0 log reduction in TVC. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^4 cfu/cm^2, and after 30 seconds Cold Jet application the TVC per cm^2 tile is < 10 cfu/cm^2(Figure 5.6).

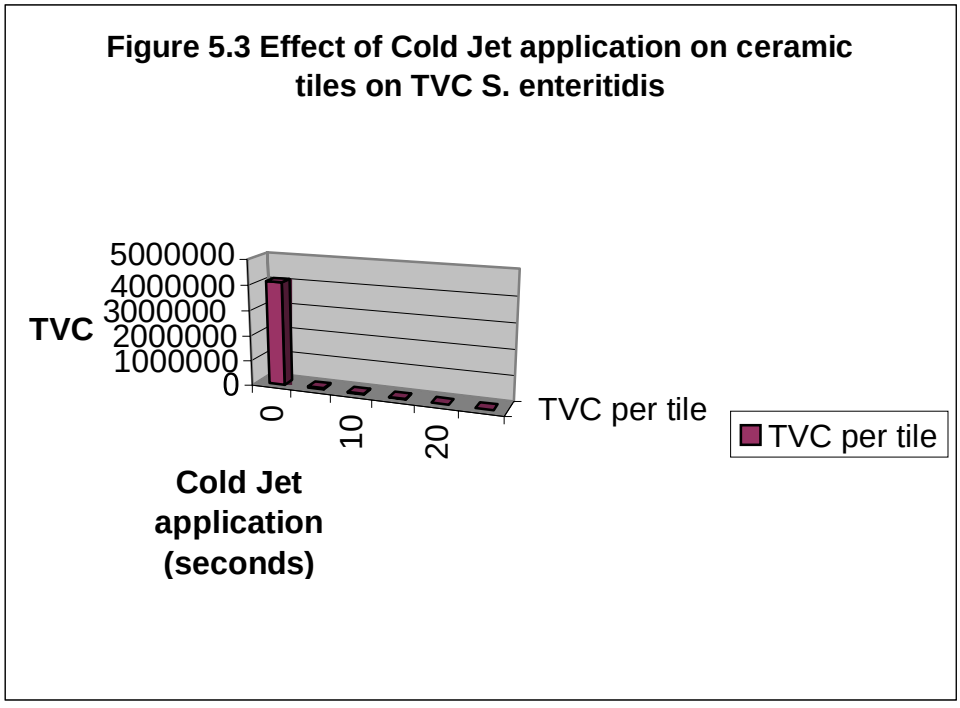
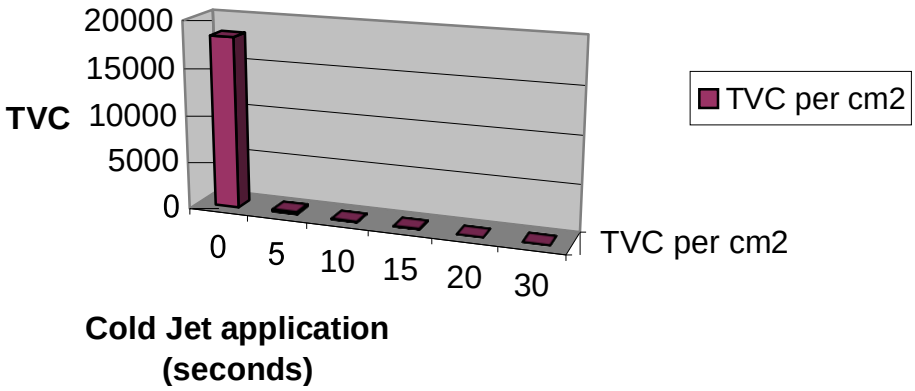
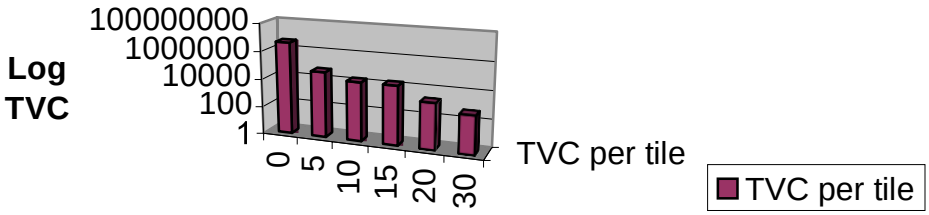


Figure 5.4 Effect of application of Cold Jet on ceramic tiles on TVC S. enteritidis



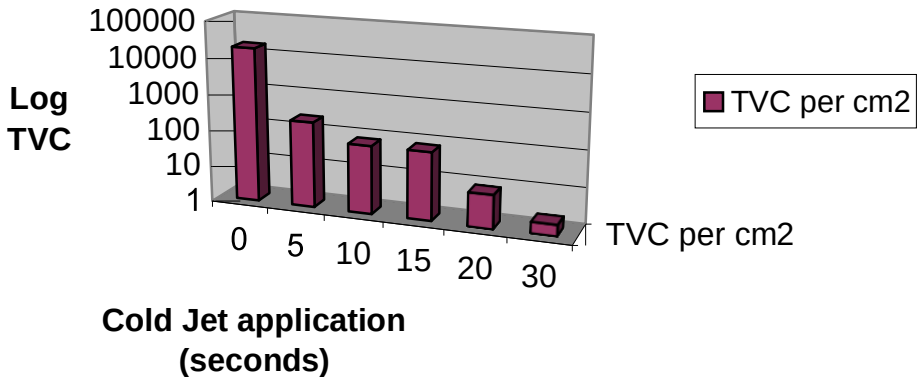
Cold Jet application (seconds)

Figure 5.5 Effect of Cold Jet application on ceramic tiles on TVC S. enteritidis



Cold Jet application (seconds)

Figure 5.6 Effect of application of Cold Jet on ceramic tiles on TVC S. enteritidis

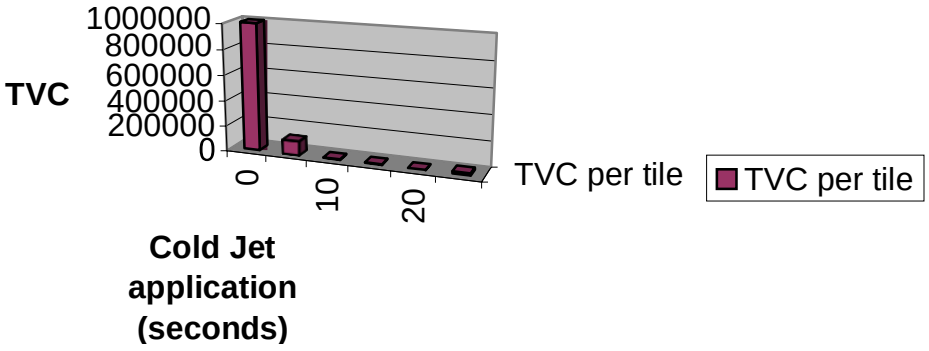


Cold Jet application (seconds)

5.3 Effect of Cold Jet treatment on plain red quarry tiles seeded with *S. enteritidis*

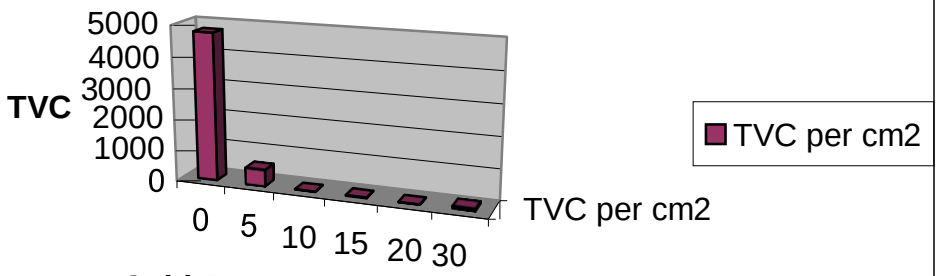
The effect of different Cold Jet application times was determined by treating identical plain red quarry tiles seeded with *S. enteritidis*. These tiles were chosen as they are typical of the sort found on floors in a wide variety of food processing operations, both large and small. Tiles (14.5cm^2) were subjected to blasting at either 0, 5, 10, 15, 20 or 30 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 30. The entire surface of the coated side of the tiles (the side that would face outwards from a surface to which the tile was fixed) was swabbed either before or after Cold Jet treatment. The results are shown in Figures 5.7 - 5.10 (Raw data are supplied in Appendix C) and show both the TVC's per tile and the TVC per cm^2 tile. The results also show a reduction in TVC's (Figures 5.7 - 5.10), but not as large as that found for plain ceramic tiles (Figures 5.3 - 5.6). After 5 seconds Cold Jet application a 1.0 log reduction was obtained (Figure 5.10) and after 30 seconds Cold Jet application a decrease of approximately 2.0 log was obtained. This was less of a reduction than that seen with plain white ceramic tiles (Section 5.2) and may be due to the porous surface on these tiles when compared with the smooth surface of the ceramic tiles, with the bacteria being present both on and underneath the surface, with the CO_2 pellets unable to penetrate into the pores of the quarrytiles.

Figure 5.7 Effect of application of Cold Jet on quarry tiles on TVC S. enteritidis



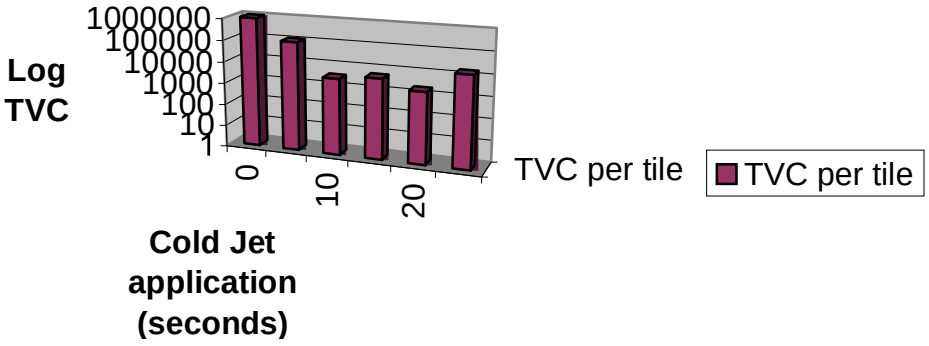
Cold Jet application (seconds)

Figure 5.8 Effect of application of Cold Jet on quarry tiles on TVC S. enteritidis



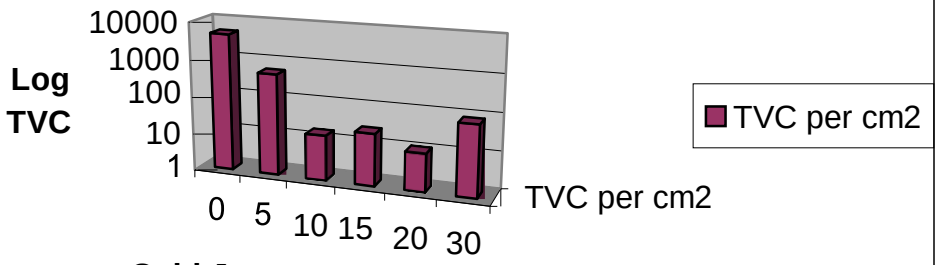
Cold Jet application (seconds)

Figure 5.9 Effect of application of Cold Jet on quarry tiles on TVC S. enteritidis



Cold Jet application (seconds)

Figure 5.10 Effect of application of Cold Jet on quarry tiles on TVC S. enteritidis



Cold Jet application (seconds)

5.4 Effect of Cold Jet treatment on plain white ceramic tiles seeded with *E. coli*

The effect of different Cold Jet application times was determined by treating identical plain white ceramic tiles seeded with *E. coli*. Tiles were subjected to blasting at either 0, 5, 10, 15, 20 or 30 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 30. The results are shown in Figures 5.11 - 5.14 (Raw data are supplied in Appendix D) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is a 2.0 log reduction in the TVC after 5 seconds, and with 30 seconds of Cold Jet application there is a 4.0 - 5.0 log reduction in TVC. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^3 cfu/cm^2, and after 30 seconds Cold Jet application the TVC per cm^2 tile is < 10 cfu/cm^2 (Figure 5.14)

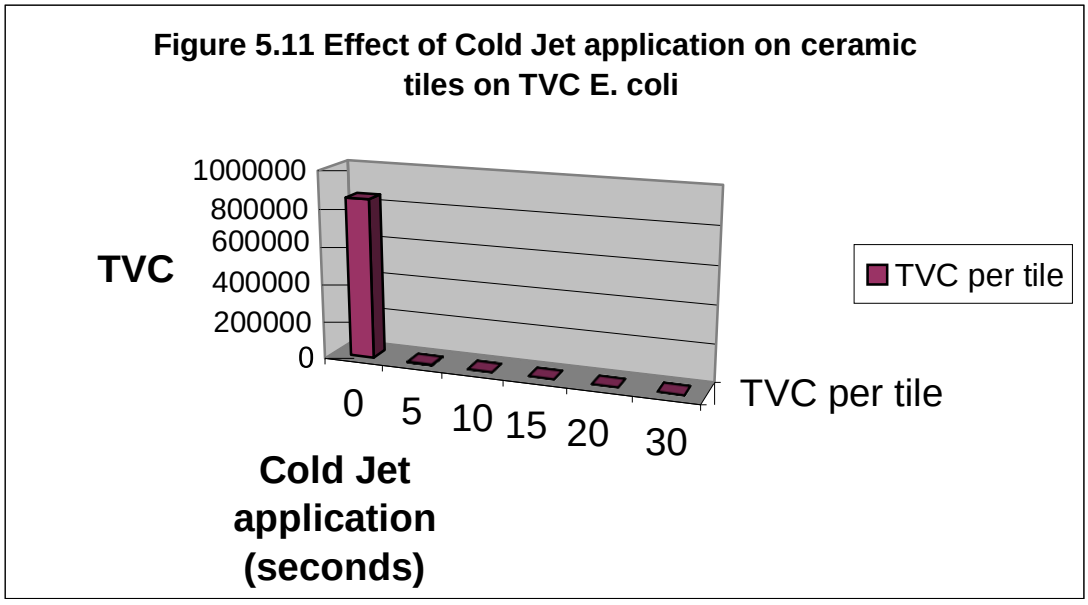


Figure 5.12 Effect of Cold Jet application on ceramic tiles on TVC E. coli

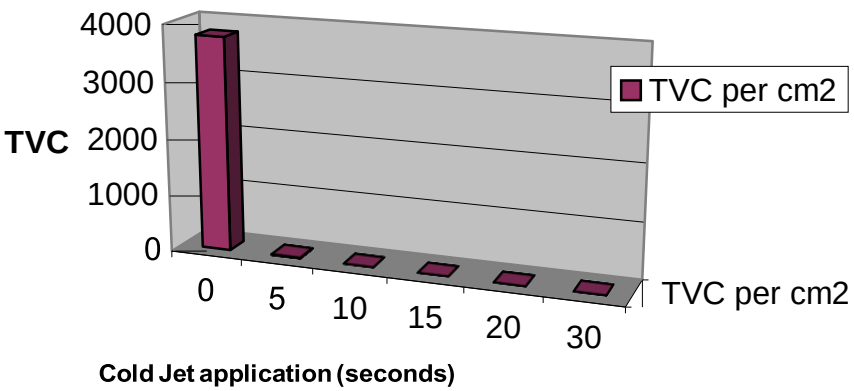


Figure 5.13 Effect of Cold Jet application on ceramic tiles on TVC E. coli

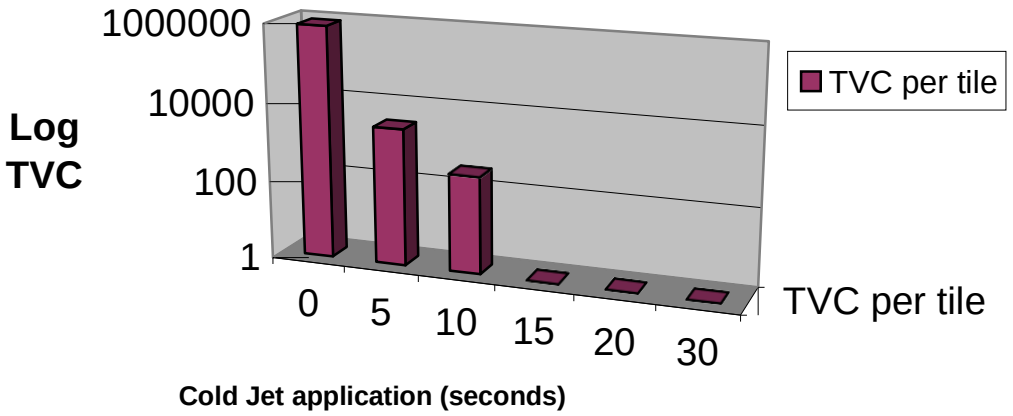
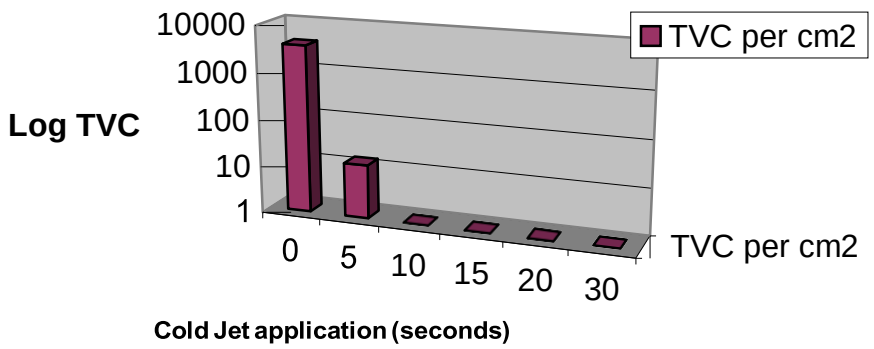


Figure 5.14 Effect of Cold Jet application on ceramic tiles on TVC E. coli



5.5 Effect of Cold Jet treatment on plain red quarry tiles seeded with *E. coli*

The effect of different Cold Jet application times was determined by treating identical plain red quarry tiles seeded with *E. coli*. Tiles were subjected to blasting at either 0, 5, 10, 15, 20 or 30 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 30. The results are shown in Figures 5.15 - 5.18 (Raw data are supplied in Appendix E) and show both the TVC's per tile and the TVC per cm^2 tile. The results also show a reduction in TVC's (Figures 5.15 - 5.18) that were generally similar to those obtained with plain ceramic tiles, with a 2.0 log reduction in TVC after 5.0 seconds treatment. There were virtually no bacteria recoverable after 15 and 20 seconds treatment - a 4.0 log reduction, but with 30 seconds treatment there was a very slight increase in TVC from 0 cfu/cm^2 to 1 cfu/ cm^2 (200 cfu/tile; Appendix E) However the TVC's are very low compared to the starting values (Figures 5.15 - 5.18; Appendix E), and at least a 2.0 log reduction in *E. coli* was found when using Cold Jet on plain red quarry tiles. There was less of a difference between Cold Jet treatments on *E. coli* whether on plain ceramic tiles or quarry tiles (Sections
5.4 & 5.5) than was found for *S. enteritidis* (Sections 5.2 & 5.3). It may be that this particular strain of *E. coli* is more susceptible to Cold Jet treatment than either *S. enteritidis* (Sections 5.2 & 5.3), or *L. monocytogenes* (Sections 5.6 & 5.7).

Figure 5.15 Effect of Cold Jet application on quarry tiles on TVC E. coli

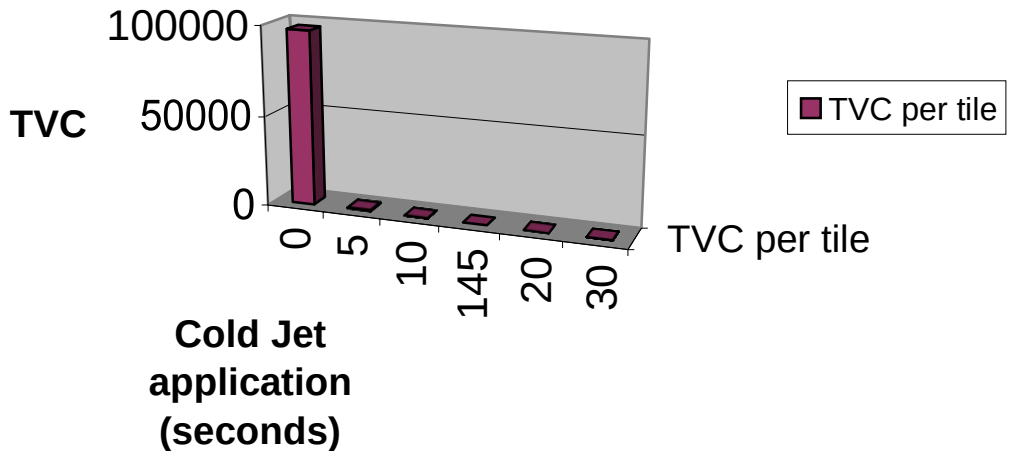


Figure 5.16 Effect of Cold Jet application on quarry tiles on TVC E. coli

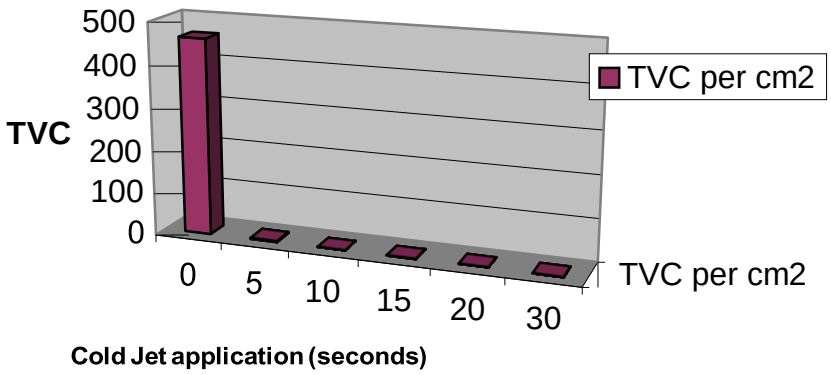


Figure 5.17 Effect of Cold Jet application on quarry tiles on TVC E. coli

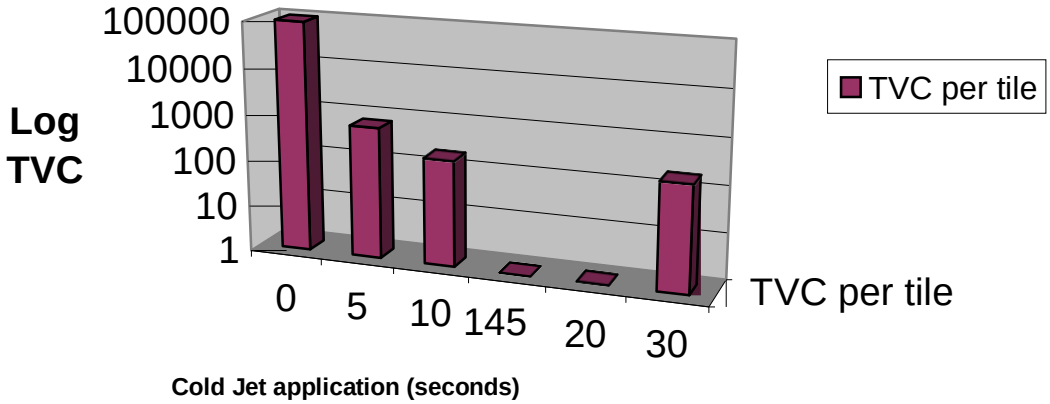
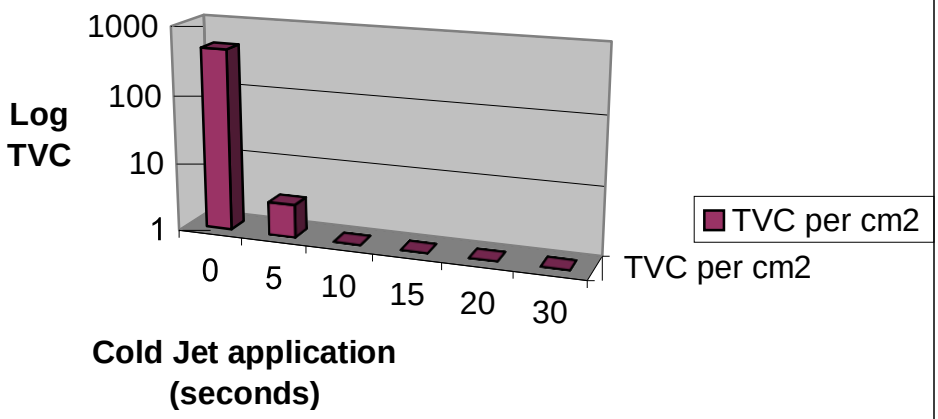


Figure 5.18 Effect of Cold Jet application on quarry tiles on TVC E. coli



5.6 Effect of Cold Jet treatment on plain white ceramic tiles seeded with *L. monocytogenes*

The effect of different Cold Jet application times was determined by treating identical plain white ceramic tiles seeded with *L. monocytogenes*. Tiles were subjected to blasting at either 0, 5, 10, 15, 20 or 30 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 30. The results are shown in Figures 5.19 - 5.22 (Raw data are supplied in Appendix F) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is an approximately 2.0 log reduction in the TVC after 5 seconds, and with 30 seconds of Cold Jet application there is a 3.0 log reduction in TVC. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^3 cfu/cm^2, and after 30 seconds Cold Jet application the TVC per cm^2 tile is < 10 cfu/cm^2(Figure 5.22).

Figure 5.19 Effect of Cold Jet application on ceramic tiles on TVC L. monocytogenes

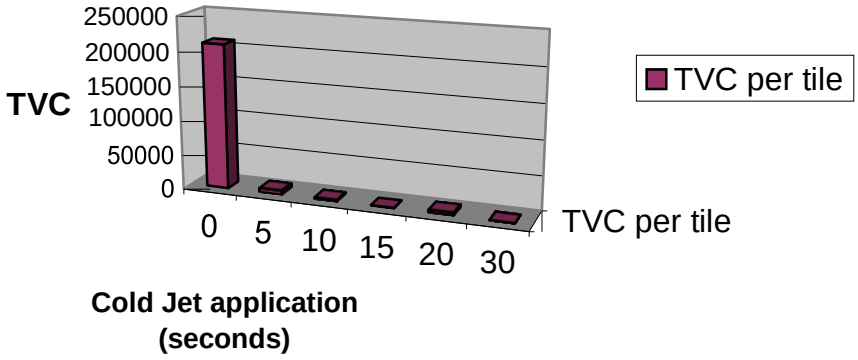


Figure 5.20 Effect of Cold Jet application on ceramic tiles on L. monocytogenes

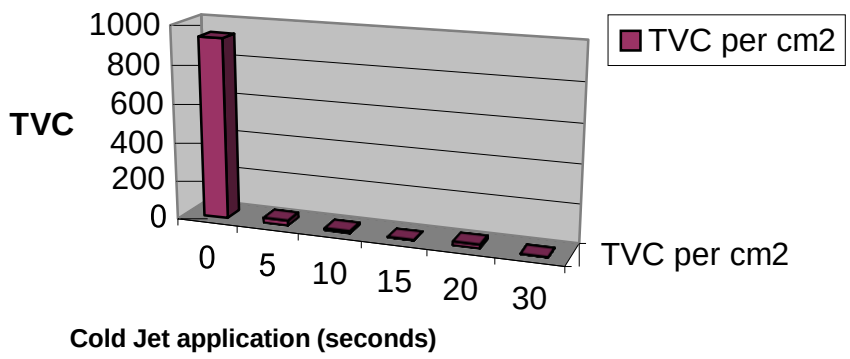


Figure 5.21 Effect of Cold Jet application on ceramic tiles on TVC L. monocytogenes

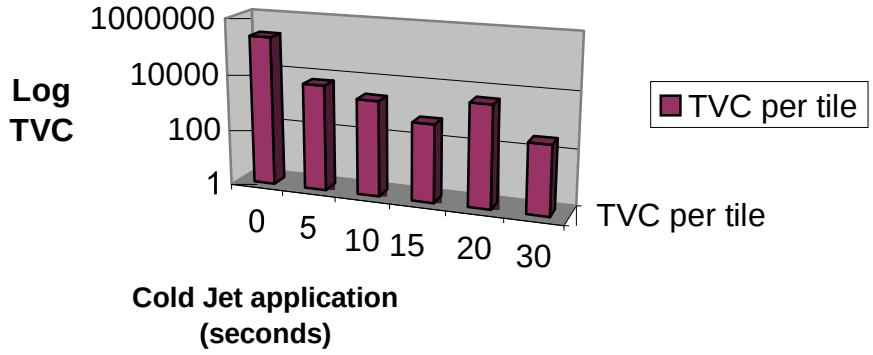
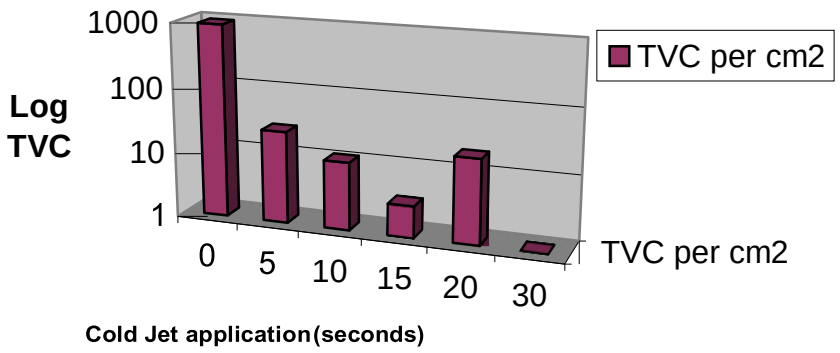


Figure 5.22 Effect of Cold Jet application on ceramic tiles on L. monocytogenes



5.7 Effect of Cold Jet treatment on plain red quarry tiles seeded with *L. monocytogenes*

The effect of different Cold Jet application times was determined by treating identical plain red quarry tiles seeded with *L. monocytogenes*. Tiles were subjected to blasting at either 0, 5, 10, 15, 20 or 30 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 30. The results are shown in Figures 5.23 - 5.26 (Raw data are supplied in Appendix G) and show both the TVC's per tile and the TVC per cm^2 tile. The results also show a reduction in TVC's (Figures 5.23 - 5.26) that were generally similar to those obtained with plain ceramic tiles (Figures 5.19 - 5.22), with a 2.0 log reduction in TVC after 5.0 seconds treatment, and only very low numbers of bacteria recoverable after 30 seconds treatment - a 3.0 log reduction (Figures 5.23 - 5.26). There results for Cold Jet treatments on *L. monocytogenes* whether on plain ceramic tiles or quarry tiles (Sections 5.6 & 5.7) were generally similar to those found for *S. enteritidis* (Sections 5.2 & 5.3), with a few hundred viable cells recovered after 30 seconds Cold Jet treatment, whereas with *E. coli* almost no viable cells were recoverable after 30 seconds treatment (Sections 5.4 & 5.5). The starting levels for all three test microorganisms applied varied from 461 - 18222 cfu/cm^2 yet at least a 2.0 log reduction in TVC of these microorganisms was found on these surfaces when treated with Cold Jet.

Figure 5.23 Effect of Cold Jet application on quarry tiles on TVC L. monocytogenes

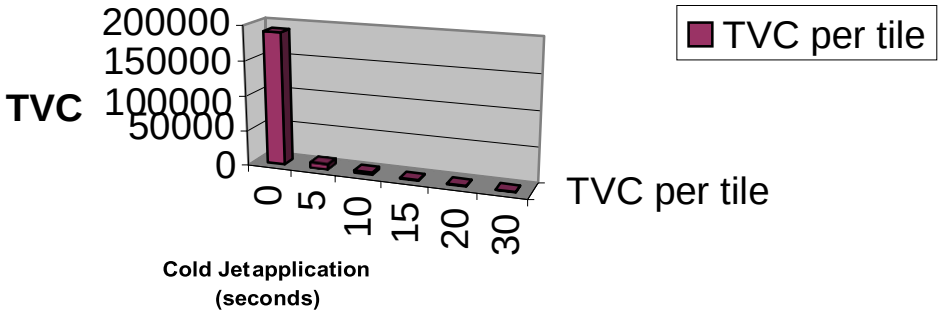


Figure 5.24 Effect of Cold Jet application on quarry tiles on TVC L. monocytogenes

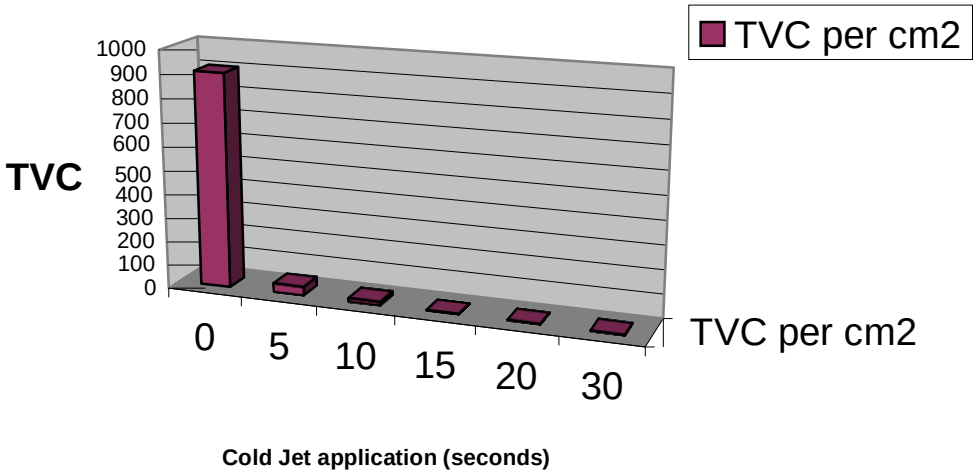


Figure 5.25 Effect of Cold Jet application on quarry tiles on TVC L. monocytogenes

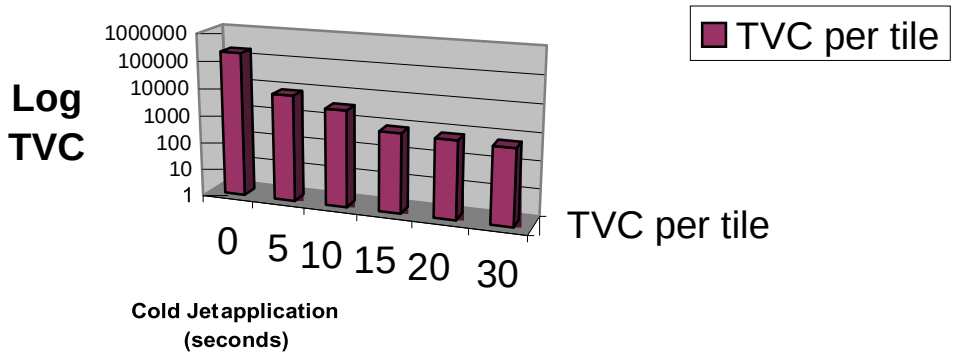
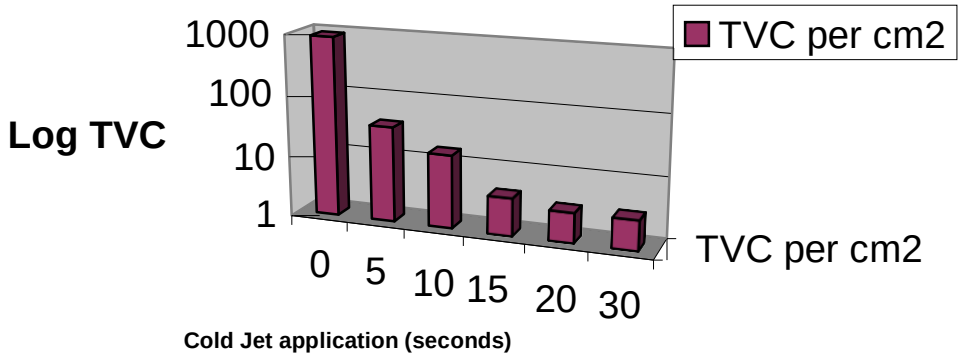


Figure 5.26 Effect of Cold Jet application on quarry tiles on TVC L. monocytogenes



5.8 Effect of Cold Jet treatment on plain white ceramic tiles seeded with *S. enteritidis*: Effect of increased CO_2 pellet feeder rate

The effect of an increase in the pellet feeder rate of Cold Jet applications was determined by treating identical plain white ceramic tiles seeded with *S. enteritidis*, which was chosen as a representative Gram negative foodborne bacteria for these experimental trials. Tiles were subjected to blasting at either 0, 5, 10, or 20 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 60 (rather than 30). The results are shown in Figures 5.27 - 5.30 (Raw data are supplied in Appendix H) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is a 2.0 log reduction in the TVC after 5 seconds, and with 20 seconds of Cold Jet application the reduction in TVC is still 2.0 log. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^3 cfu/cm^2, and after 20 seconds Cold Jet application the TVC per cm^2 tile is approximately 10 cfu/cm^2 (Figure 5.30). It can be seen that increasing the pellet feeder rate made no difference to the level of viable *S. enteritidis* recovered (see Section 5.2). The maximum application of Cold Jet was 20 seconds due to the increased rate of pellet usage and also that after 20 seconds the pellets were forming a layer on the surface due to the surface becoming extremely cold. Therefore, a maximum Cold Jet application time of 20 seconds was used for all subsequent experimental trials (see Sections 5.9 - 5.11).

Figure 5.27 Effect of Cold Jet (PFR=60) application on ceramic tiles on TVC S. enteritidis

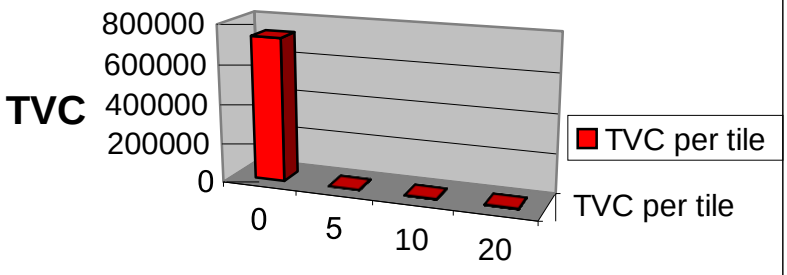


Figure 5.28 Effect of Cold Jet (PFR = 60) application on ceramic tiles on TVC S. enteritidis

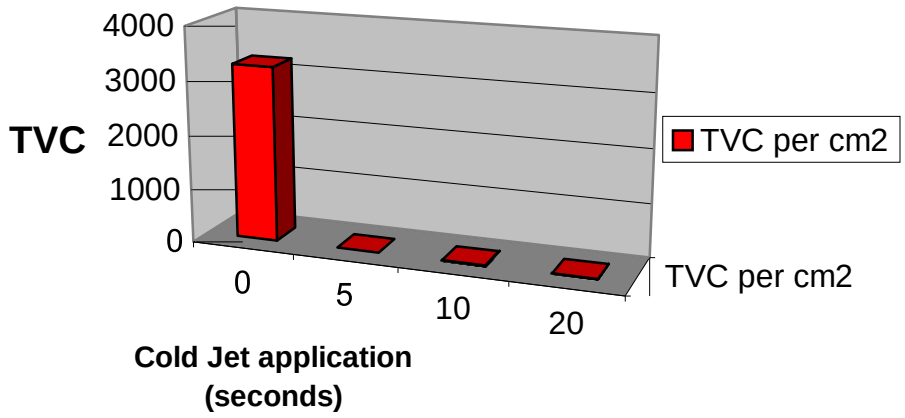


Figure 5.29 Effect of Cold Jet (PFR=60) application on ceramic tiles on TVC S. enteritidis

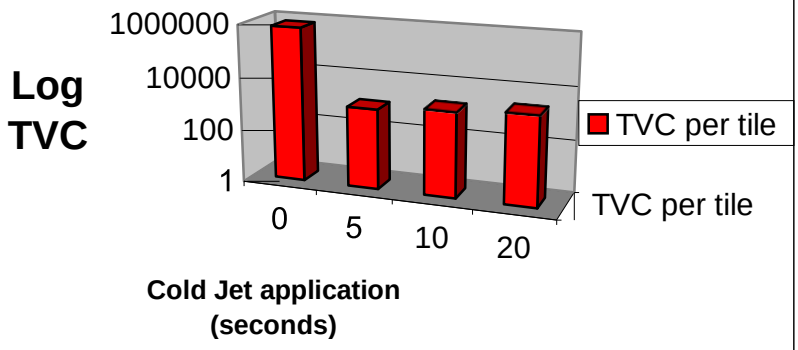
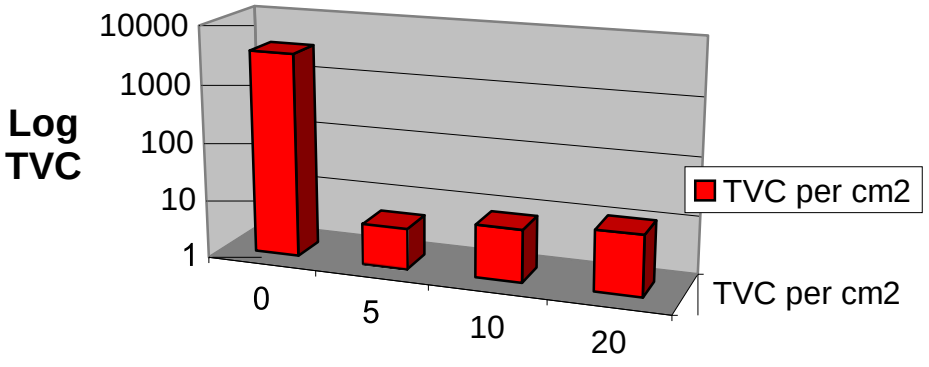


Figure 5.30 Effect of Cold Jet (PFR = 60) application on ceramic tiles on TVC S. enteritidis



5.9 Effect of Cold Jet treatment on plain red quarry tiles seeded with *S. enteritidis*: Effect of increased CO_2 pellet feeder rate

The effect of an increase in the pellet feeder rate of Cold Jet applications was determined by treating identical plain red quarry tiles seeded with *S. enteritidis*, which was chosen as a representative Gram negative foodborne bacteria for these experimental trials. Tiles were subjected to blasting at either 0, 5, 10, or 20 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 60 (rather than 30). The results are shown in Figures 5.31 - 5.34 (Raw data are supplied in Appendix I) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is a 2.0 log reduction in the TVC after 5 seconds, and with 20 seconds of Cold Jet application the reduction in TVC is still 2.0 log. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^3 cfu/cm^2, and after 20 seconds Cold Jet application the TVC per cm^2 tile is approximately 10 cfu/cm^2 (Figure 5.34). As with the plain ceramic tiles (Section 5.8), increasing the pellet feeder rate made no difference to the level of viable *S. enteritidis* recovered (see Section 5.3).

Figure 5.31 Effect of Cold Jet application (PFR = 60) on quarry tiles on TVC S. enteritidis

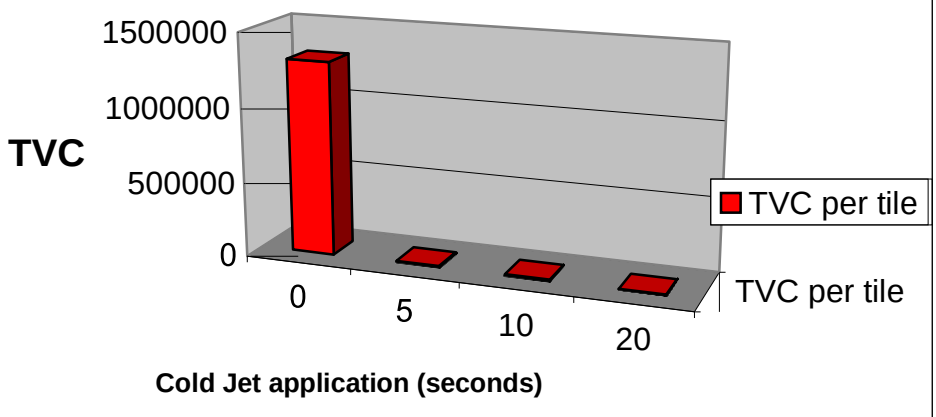


Figure 5.32 Effect of Cold Jet (PFR = 60) application on quarry tiles on TVC S. enteritidis

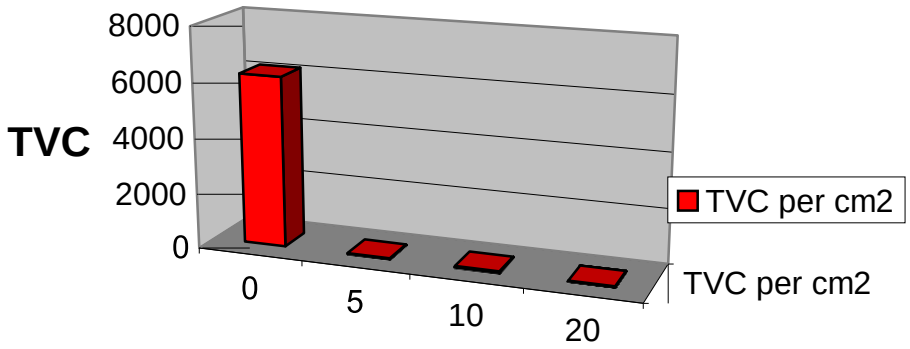


Figure 5.33 Effect of Cold Jet application (PFR = 60) on quarry tiles on TVC S. enteritidis

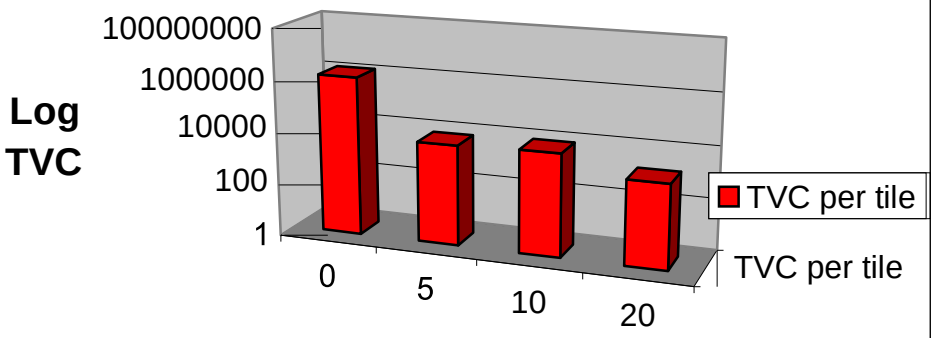
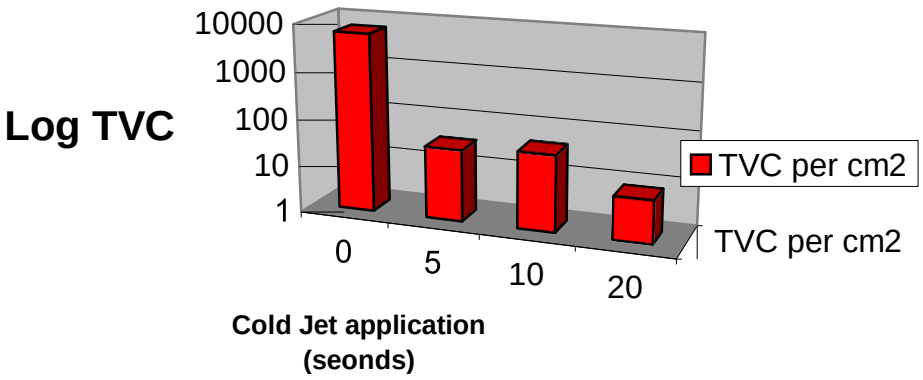


Figure 5.34 Effect of Cold Jet (PFR = 60) application on quarry tiles on TVC S. enteritidis



Cold Jet application (seonds)

5.10 Effect of Cold Jet treatment on plain white ceramic tiles seeded with *L. monocytogenes*: Effect of increased CO_2 pellet feeder rate

The effect of an increase in the pellet feeder rate of Cold Jet applications was determined by treating identical plain white ceramic tiles seeded with *L. monocytogenes*, which was chosen as a representative Gram positive foodborne bacteria for these experimental trials. Tiles were subjected to blasting at either 0, 5, 10, or 20 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 60 (rather than 30). The results are shown in Figures 5.35 - 5.38 (Raw data are supplied in Appendix J) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is a 2.0 log reduction in the TVC after 5 seconds, and with 20 seconds of Cold Jet application the reduction in TVC is still 2.0 log. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^3 cfu/cm^2, and after 20 seconds Cold Jet application the TVC per cm^2 tile is approximately < 10 cfu/cm^2 (Figure 5.38). It can be seen that increasing the pellet feeder rate made no difference to the level of viable *L. monocytogenes* recovered (see Section 5.6).

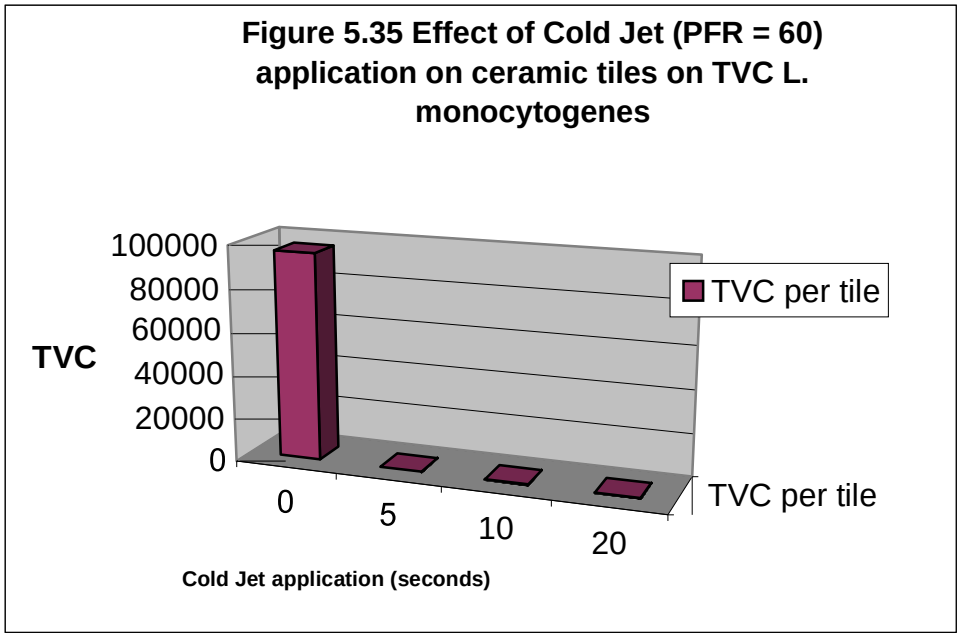


Figure 5.36 Effect of Cold Jet (PFR = 60) application on ceramic tiles on TVC L. monocytogenes

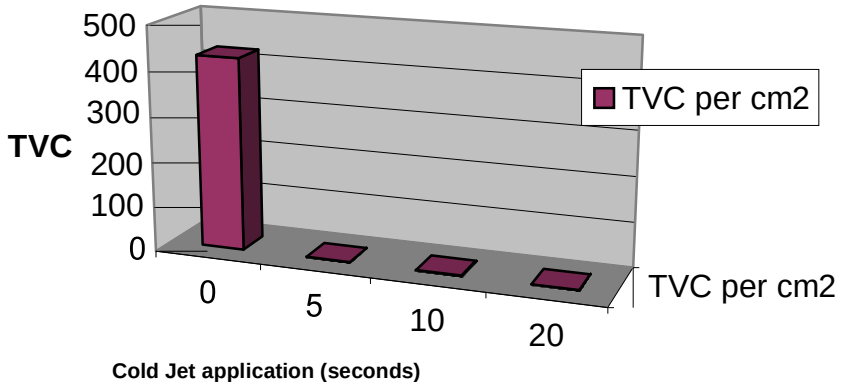
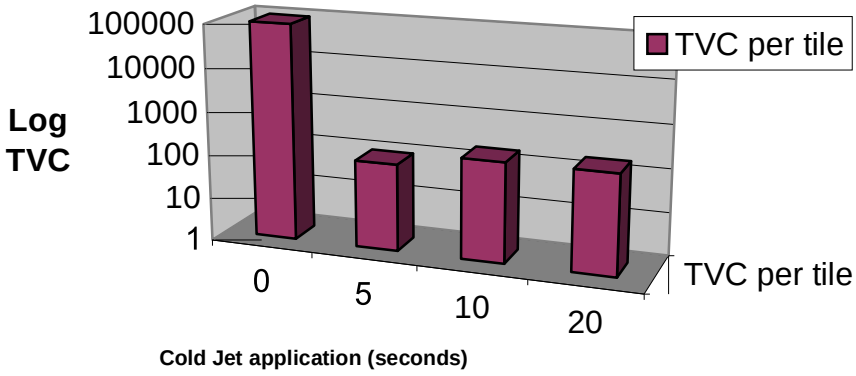
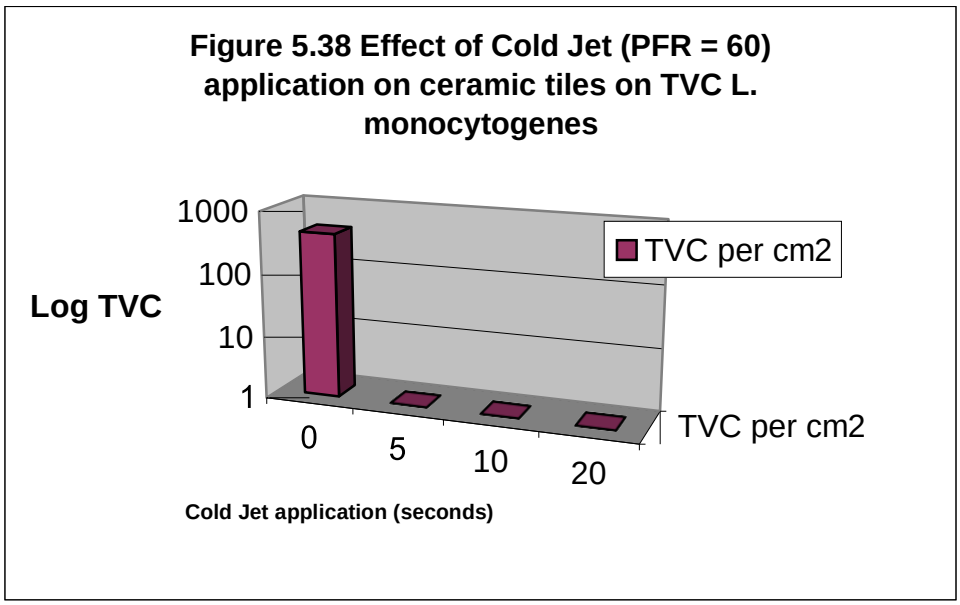


Figure 5.37 Effect of Cold Jet (PFR = 60) application on ceramic tiles on TVC L. monocytogenes





5.11 Effect of Cold Jet treatment on plain red quarry tiles seeded with *L. monocytogenes*: Effect of increased CO_2 pellet feeder rate

The effect of an increase in the pellet feeder rate of Cold Jet applications was determined by treating identical plain red quarry tiles seeded with *L. monocytogenes*, which was chosen as a representative Gram positive foodborne bacteria for these experimental trials. Tiles were subjected to blasting at either 0, 5, 10, or 20 seconds with 3mm diameter pellets at an outlet blast pressure of 40 psi and a pellet feeder rate of 60 (rather than 30). The results are shown in Figures 5.39 - 5.42 (Raw data are supplied in Appendix K) and show both the TVC's per tile and the TVC per cm^2 tile. It can be seen that there is a 2.0 log reduction in the TVC after 5 seconds, and with 20 seconds of Cold Jet application the reduction in TVC is approximately 3.0 log. Thus the TVC per cm^2 tile before Cold Jet application is approximately 10^3 cfu/cm^2, and after 20 seconds Cold Jet application the TVC per cm^2 tile is < 10 cfu/cm^2 (Figure 5.42). Increasing the pellet feeder rate made no difference to the level of viable *L. monocytogenes* recovered (see Section 5.7).

Figure 5.39 Effect of Cold Jet (PFR = 60) application on quarry tiles on TVC L. monocytogenes

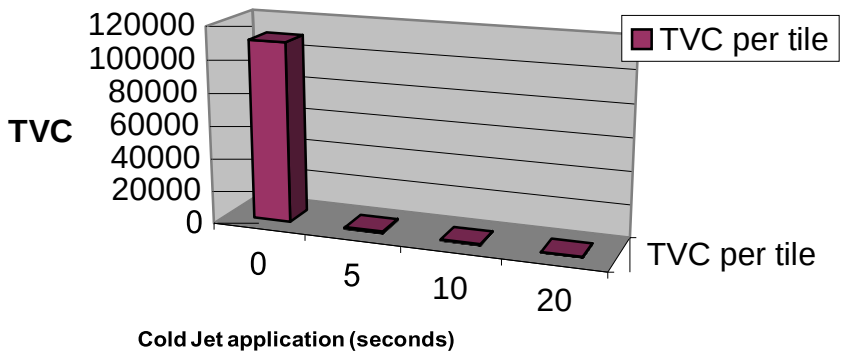


Figure 5.40 Effect of Cold Jet (PFR = 60) application on quarry tiles on TVC L. monocytogenes

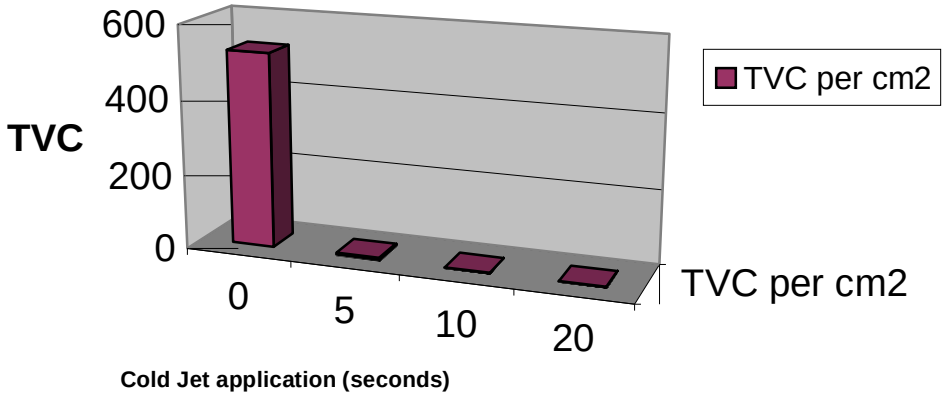


Figure 5.41 Effect of Cold Jet (PFR = 60) application on quarry tiles on TVC L. monocytogenes

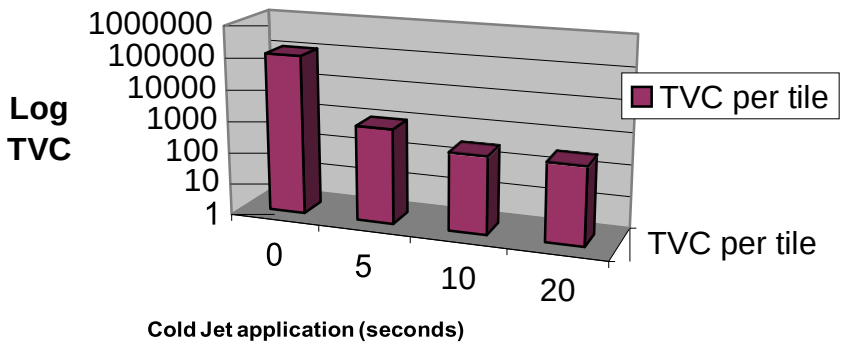
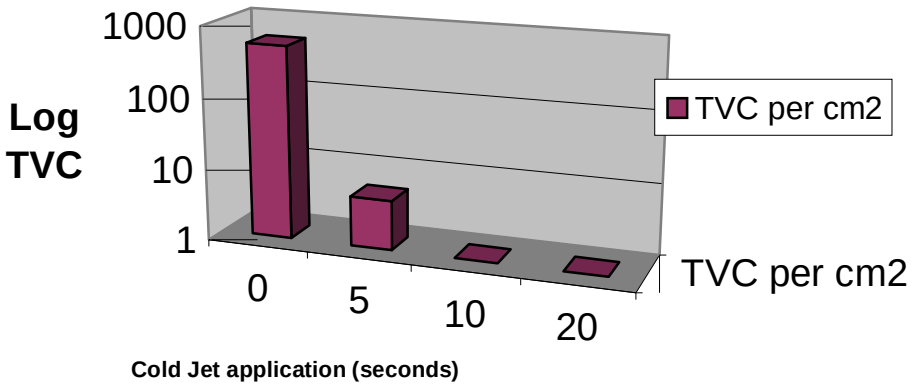


Figure 5.42 Effect of Cold Jet (PFR = 60) application on quarry tiles on TVC L. monocytogenes



5.12 Effect of treatment of surfaces with air-blasting alone

The effect of using air blasting only, i.e. passing compressed air through the Cold Jet nozzle with no CO_2 pellets in the air stream, was investigated by treating plain white ceramic tiles and plain red quarry tiles seeded with either *S. enteritidis*, *E. coli* or *L. monocytogenes*. Surfaces were subjected to air blast treatment for 0, 5 or 30 seconds with outlet blast pressures of 30 to 50 psi.

The results for *S. enteritidis* are shown in Figures 5.43 - 5.50 (Raw data are supplied in Appendix L) and show a 2.0 log reduction for ceramic tiles and a 1.5 log reduction for quarry tiles after 30 seconds treatment with air blast alone. This compares with a 3.0 - 4.0 log reduction for ceramic tiles and a 2.0 log reduction for quarry tiles when using Cold Jet with CO_2 pellets (see Sections 5.2 & 5.3).

Figure 5.43 Effect of air blast application alone on ceramic tiles on TVC S. enteritidis

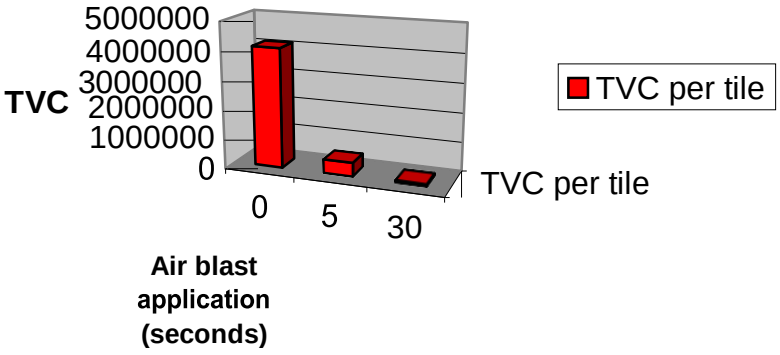


Figure 5.44 Effect of air blast application alone on ceramic tiles on TVC S. enteritidis

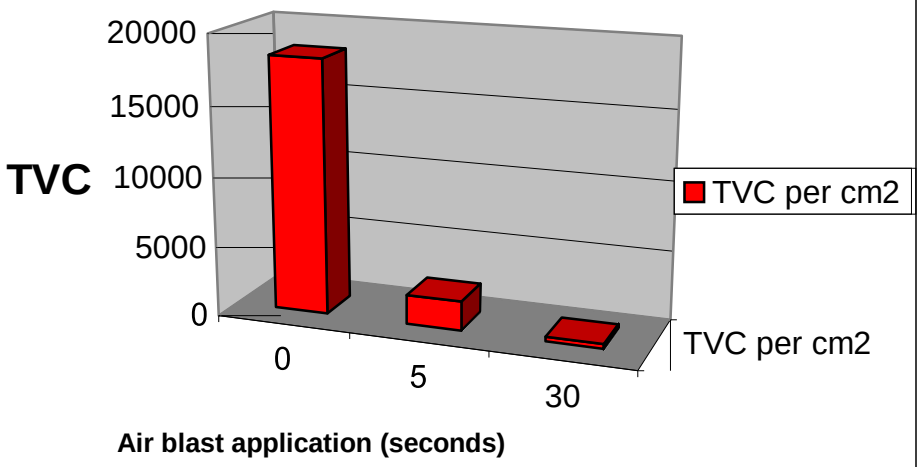


Figure 5.45 Effect of air blast application alone on ceramic tiles on TVC S. enteritidis

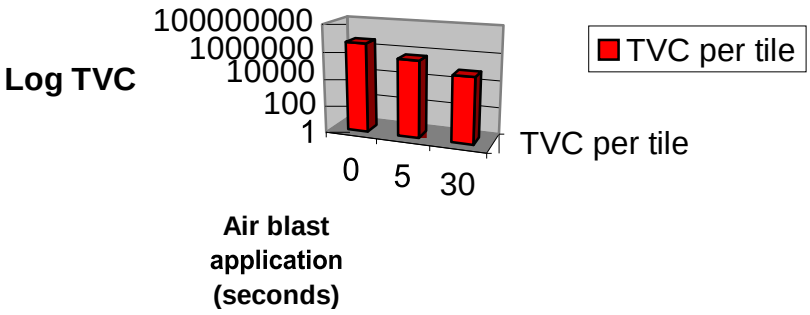


Figure 5.46 Effect of air blast application alone on ceramic tiles on TVC S. enteritidis

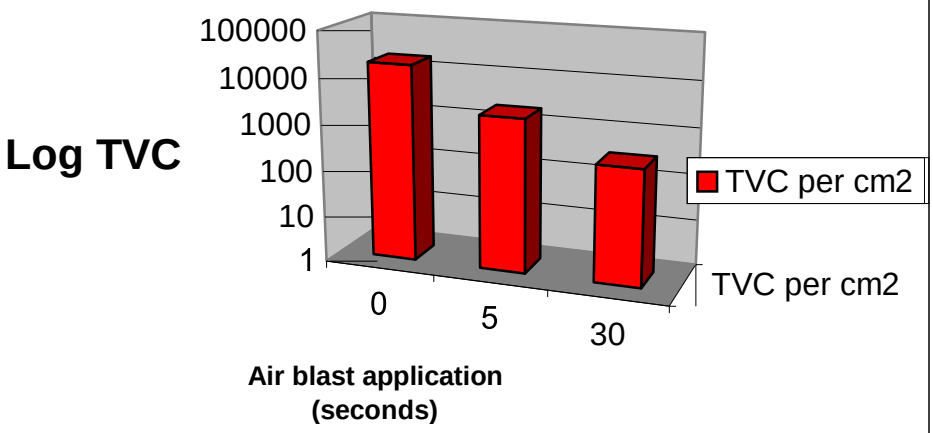


Figure 5.47 Effect of air blast application alone on quarry tiles on TVC S. enteritidis

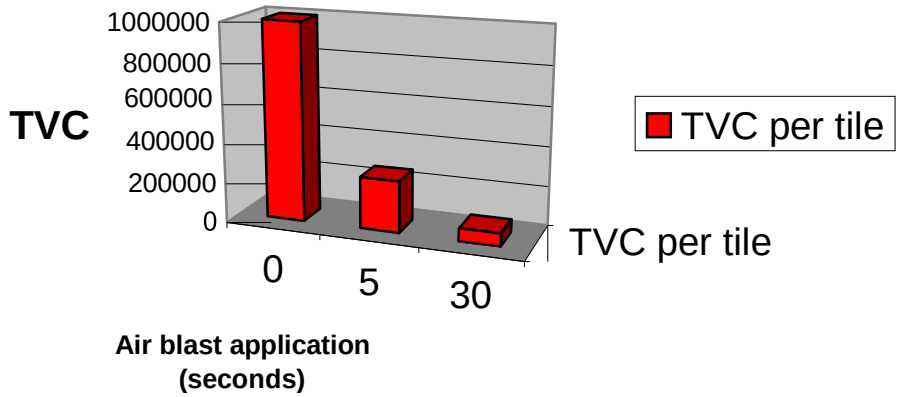


Figure 5.48 Effect of air blast application alone on quarry tiles on TVC S. enteritidis

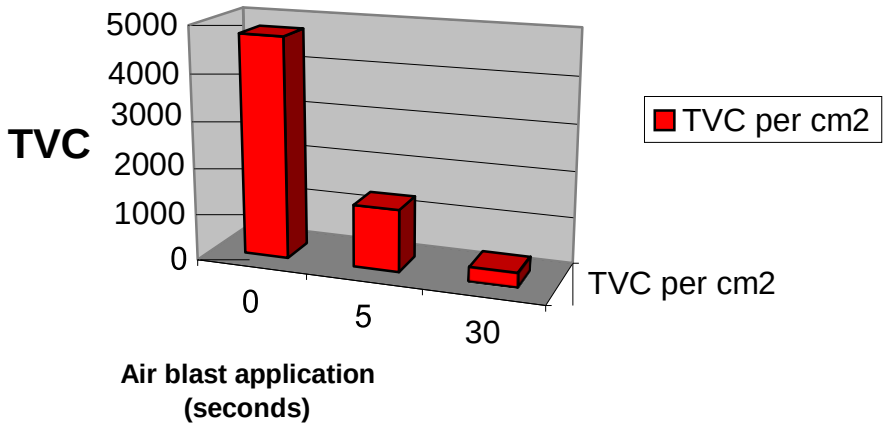


Figure 5.49 Effect of air blast application alone on quarry tiles on TVC S. enteritidis

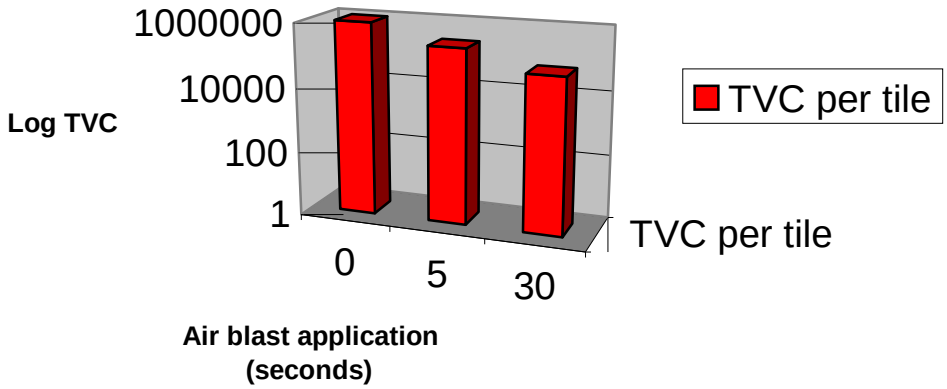
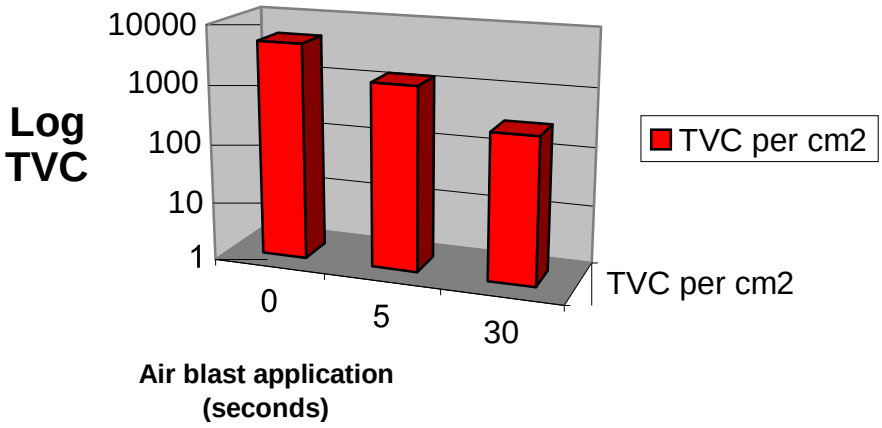


Figure 5.50 Effect of air blast application alone on quarry tiles on TVC S. enteritidis



The results for *E. coli* are shown in Figures 5.51 - 5.58 (Raw data are supplied in Appendix L) and show a 2.0 log reduction for ceramic tiles and a 2.5 - 3.0 log reduction for quarry tiles after 30 seconds treatment with air blast alone. This compares with a 4.0 - 5.0 log reduction for ceramic tiles and a 2.0 log reduction with quarry tiles when using Cold Jet with CO_2 pellets (see Sections 5.4 & 5.5).

Figure 5.51 Effect of air blast application alone on ceramic tiles on TVC E. coli

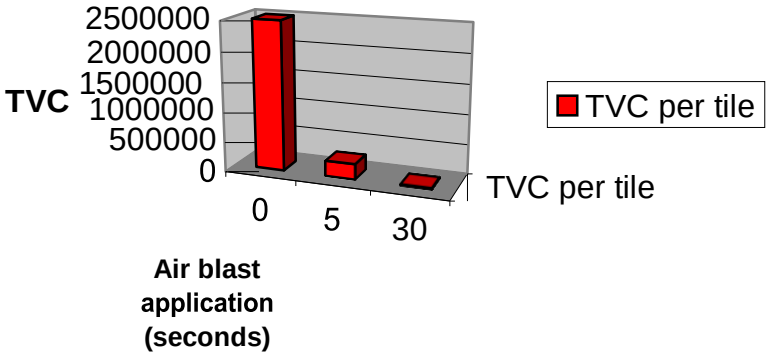


Figure 5.52 Effect of air blast application alone on ceramic tiles on TVC E. coli

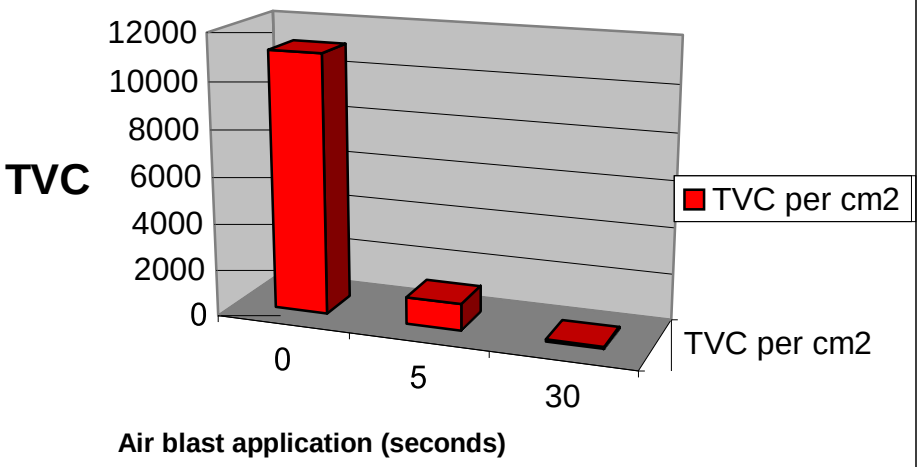


Figure 5.53 Effect of air blast application alone on ceramic tiles on TVC E. coli

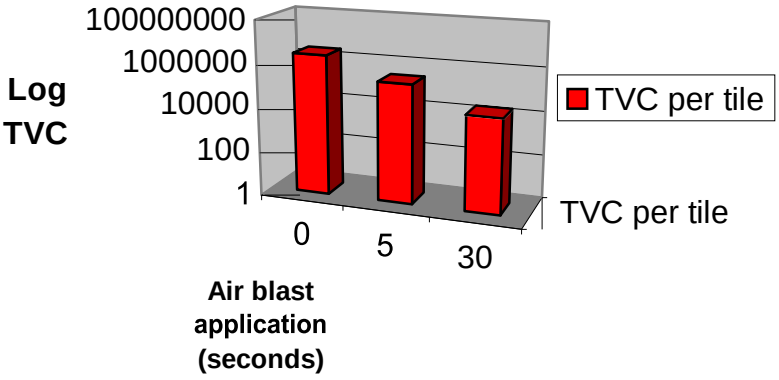


Figure 5.54 Effect of air blast application alone on ceramic tiles on TVC E. coli

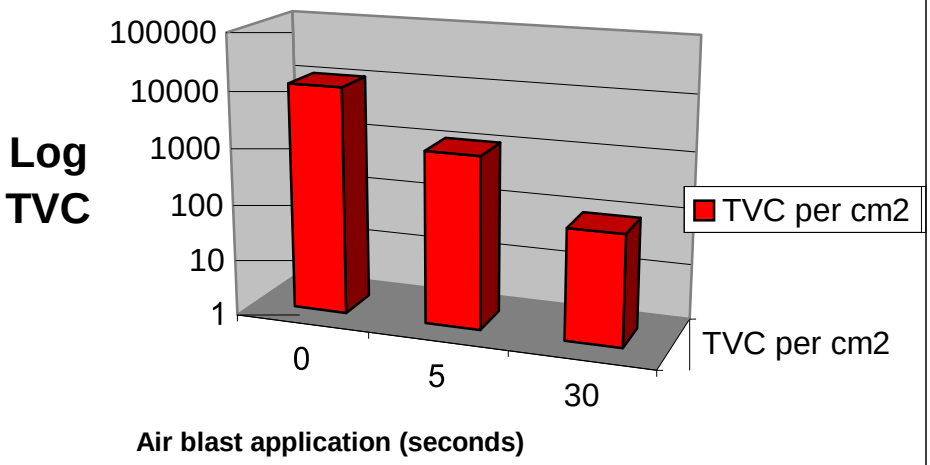


Figure 5.55 Effect of air blast application alone on quarry tiles on TVC E. coli

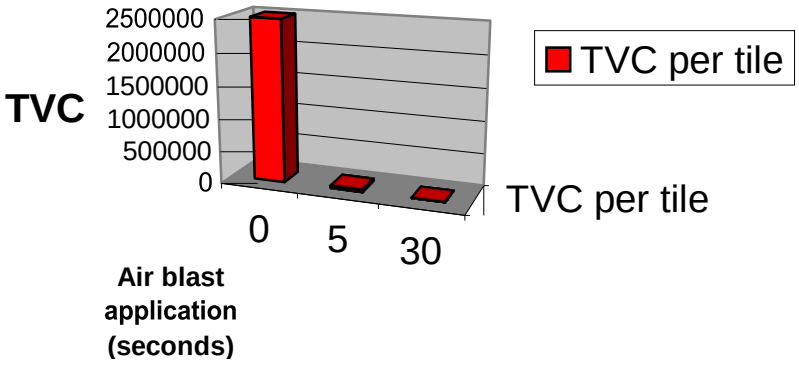


Figure 5.56 Effect of air blast application alone on quarry tiles on TVC E. coli

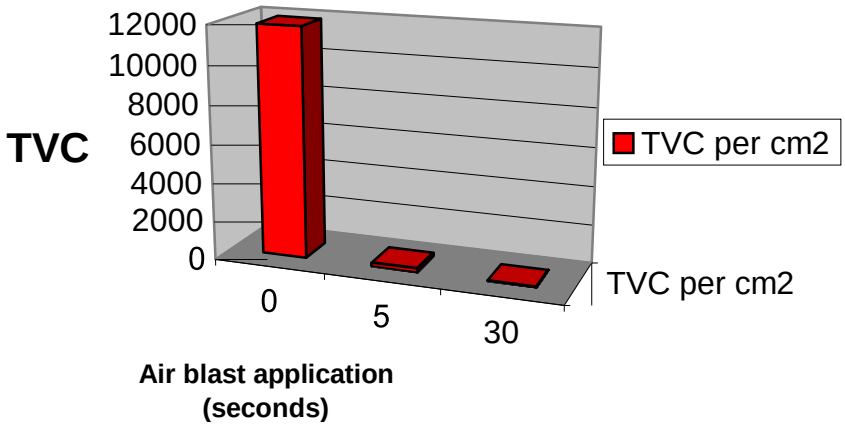


Figure 5.57 Effect of air blast application alone on quarry tiles on TVC E. coli

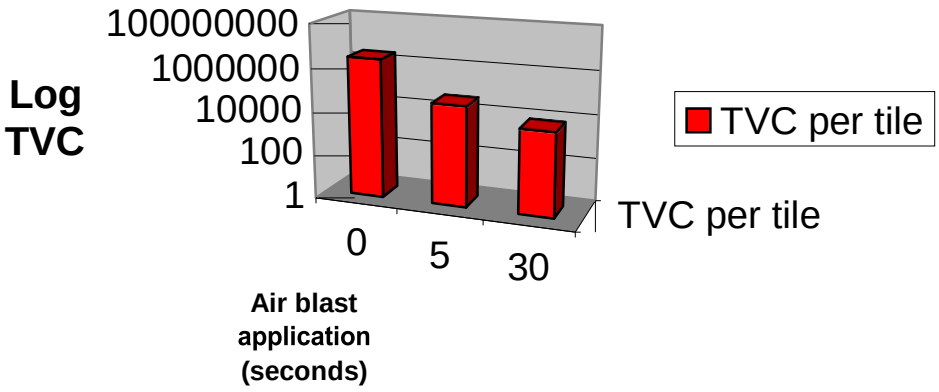
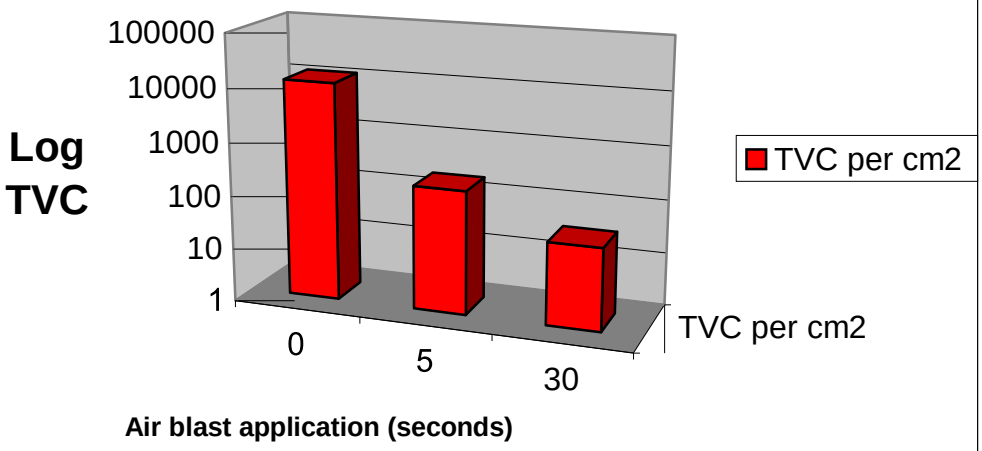


Figure 5.58 Effect of air blast application alone on quarry tiles on TVC E. coli



The results for *L. monocytogenes* are shown in Figures 5.59 - 5.66 (Raw data are supplied in Appendix L) and show a 2.0 log reduction for ceramic tiles and a 1.0 log reduction for quarry tiles after 30 seconds treatment with air blast alone. This compares with a 3.0 log reduction for ceramic tiles and a 3.0 log reduction with quarry tiles when using Cold Jet with CO_2 pellets (see Sections 5.6 & 5.7).

Figure 5.59 Effect of air blast application alone on ceramic tiles on TVC L. monocytogenes

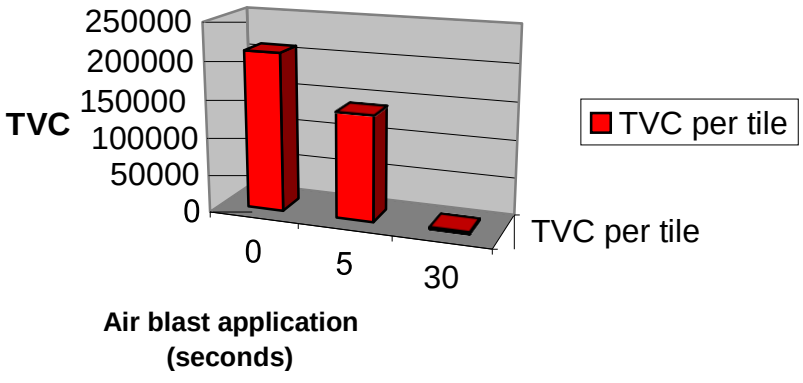


Figure 5.60 Effect of air blast application alone on ceramic tiles on TVC L. monocytogenes

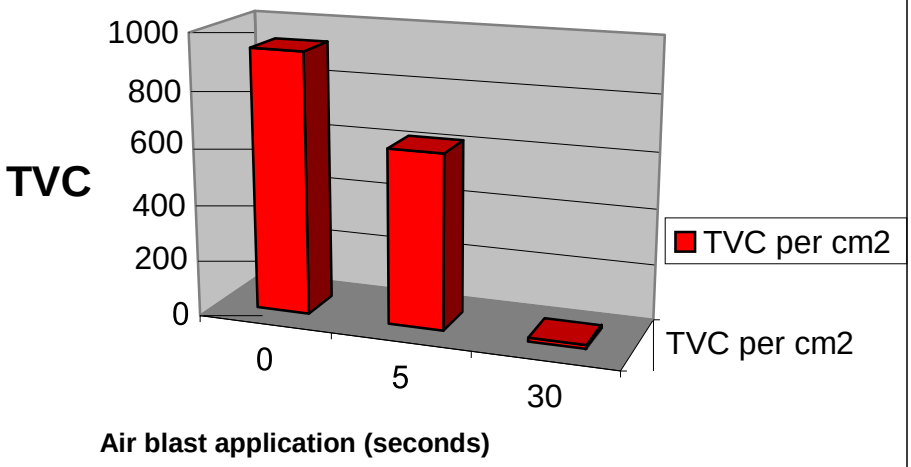


Figure 5.61 Effect of air blast application alone on ceramic tiles on TVC L. monocytogenes

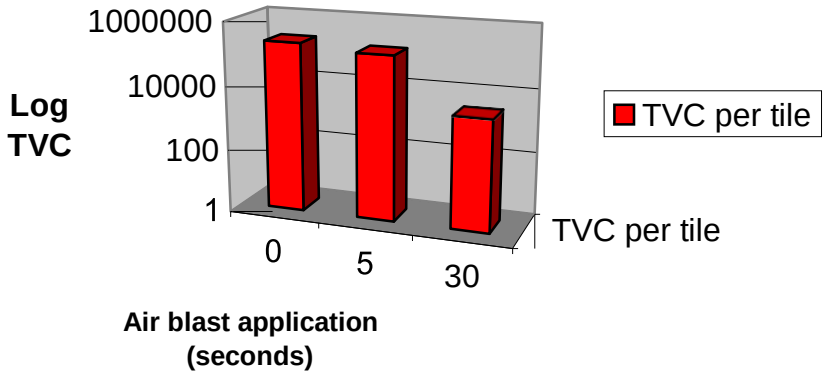


Figure 5.62 Effect of air blast application alone on ceramic tiles on TVC L. monocytogenes

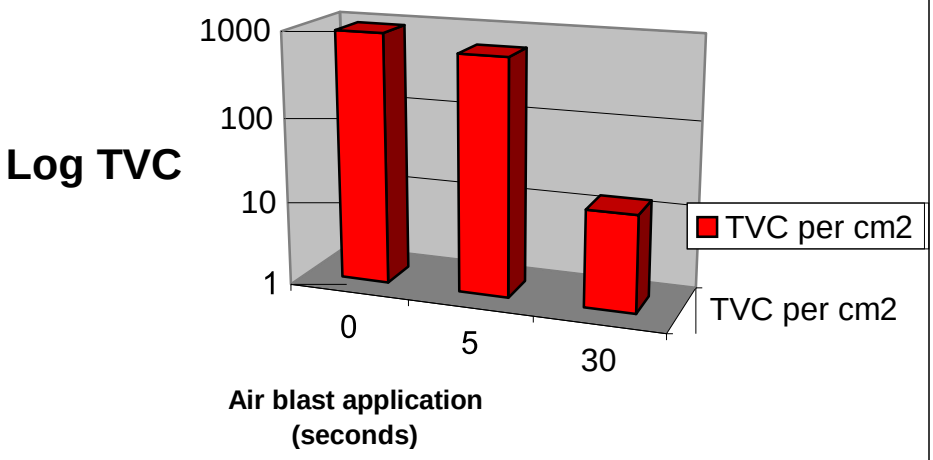


Figure 5.63 Effect of air blast application alone on quarry tiles on TVC L. monocytogenes

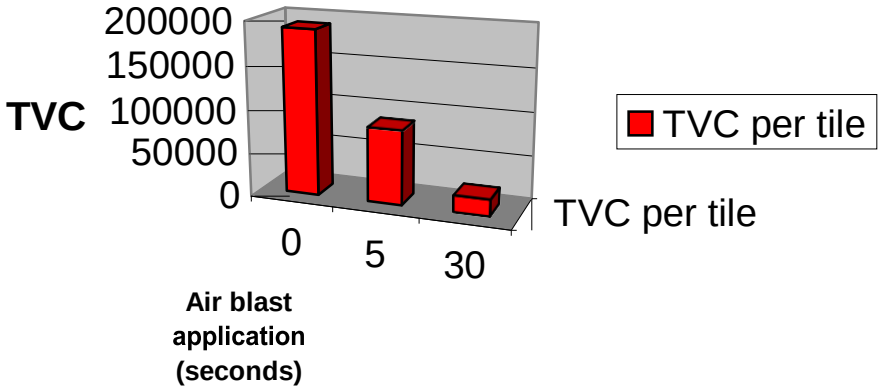


Figure 5.64 Effect of air blast application alone on quarry tiles on TVC L. monocytogenes

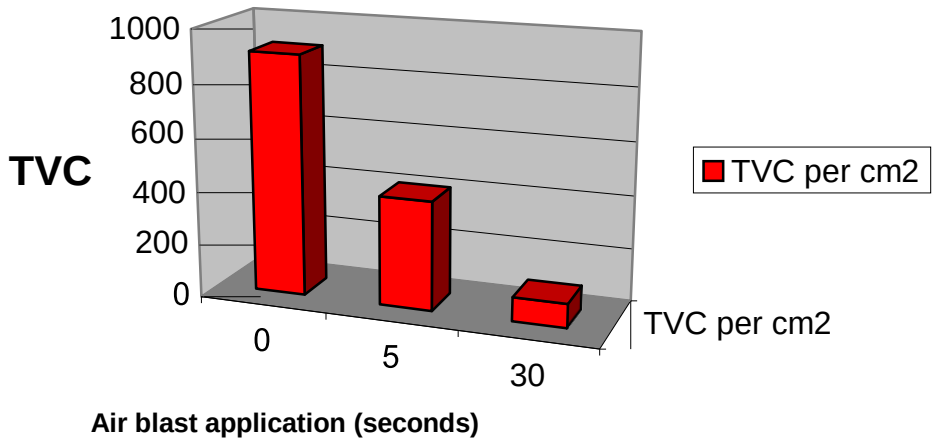


Figure 5.65 Effect of air blast application alone on quarry tiles on TVC L. monocytogenes

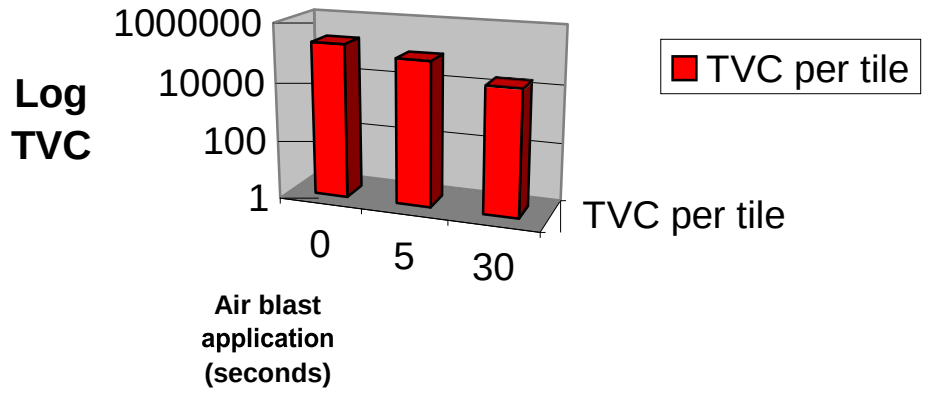
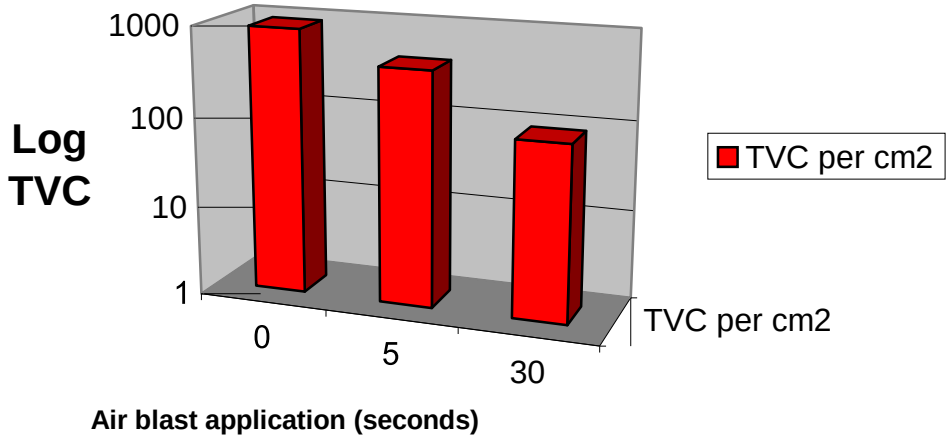


Figure 5.66 Effect of air blast application alone on quarry tiles on TVC L. monocytogenes



5.13 Effect of treatment of surfaces with CO_2 pellets alone

The effect of using CO_2 pellets only was investigated by treating plain white ceramic tiles and plain red quarry tiles seeded with either *S. enteritidis*, *E. coli* or *L. monocytogenes* with CO_2 pellets alone. Seeded surfaces were covered with CO_2 pellets for 0, 5 or 30 seconds.

The results for *S. enteritidis* are shown in Figures 5.67 - 5.74 (Raw data are supplied in Appendix M) and show a 1.5 log reduction for ceramic tiles and a 1.5 log reduction for quarry tiles after 30 seconds treatment with CO_2 pellets alone. This compares with a 3.0 - 4.0 log reduction for ceramic tiles and a 2.0 log reduction for quarry tiles when using CO_2 pellets with Cold Jet (see Sections 5.2 & 5.3).

Figure 5.67 Effect of CO2 pellet application alone on ceramic tiles on TVC S. enteritidis

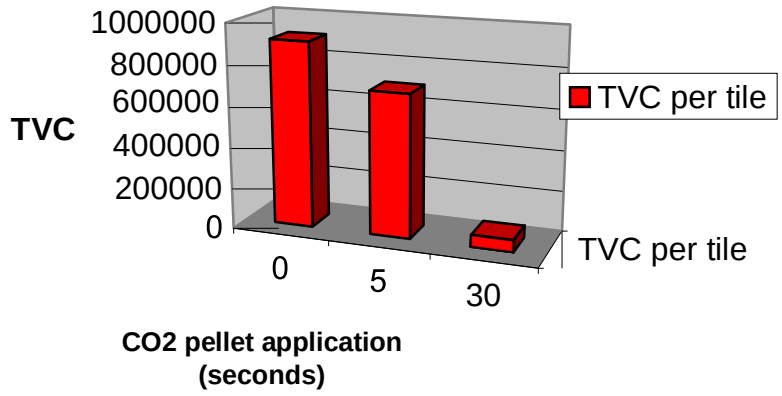


Figure 5.68 Effect of CO2 pellet application alone on ceramic tiles on TVC S. enteritidis

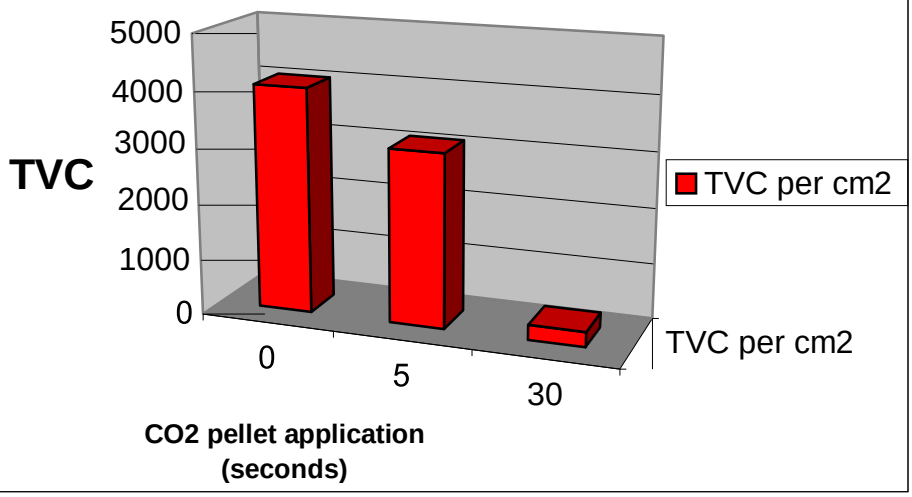


Figure 5.69 Effect of CO2 pellet application alone on ceramic tiles on TVC S. enteritidis

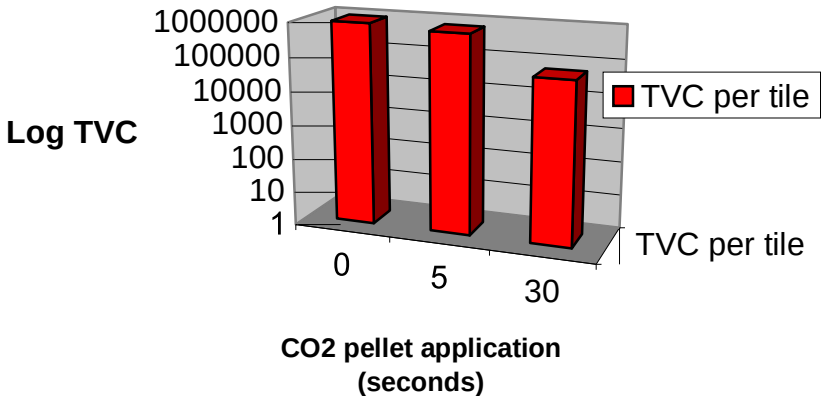


Figure 5.70 Effect of CO2 pellet application alone on ceramic tiles on TVC S. enteritidis

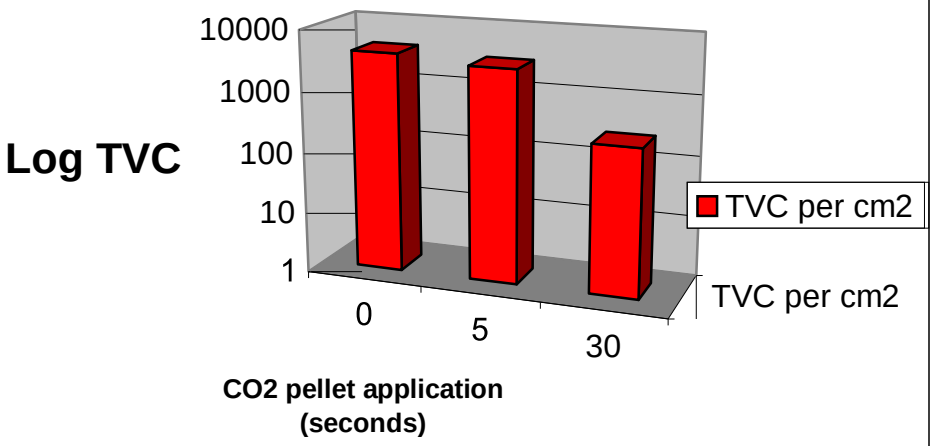


Figure 5.71 Effect of CO2 pellet application alone on quarry tiles on TVC S. enteritidis

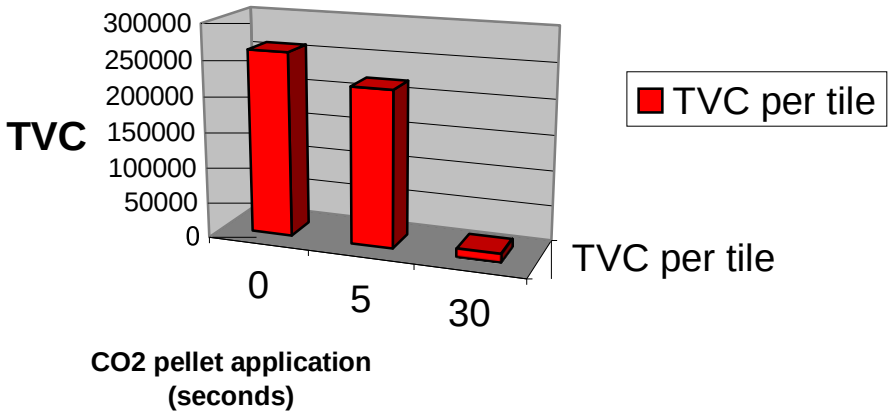


Figure 5.72 Effect of CO2 pellet application alone on quarry tiles on TVC S. enteritidis

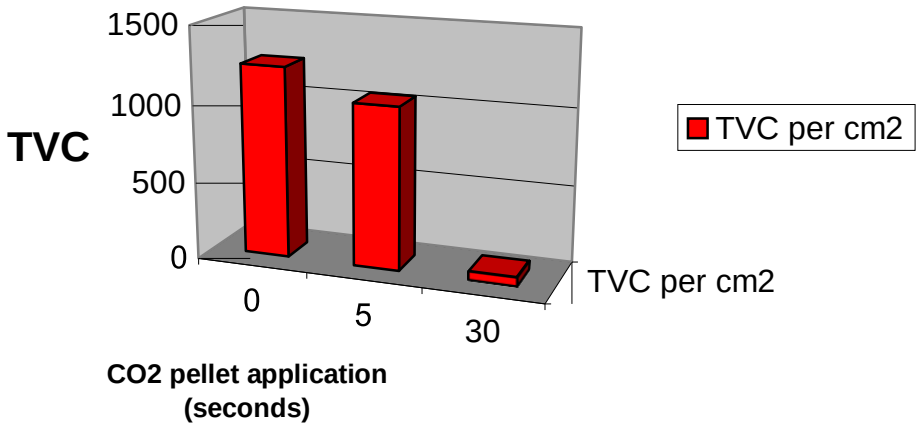


Figure 5.73 Effect of CO2 pellet application alone on quarry tiles on TVC S. enteritidis

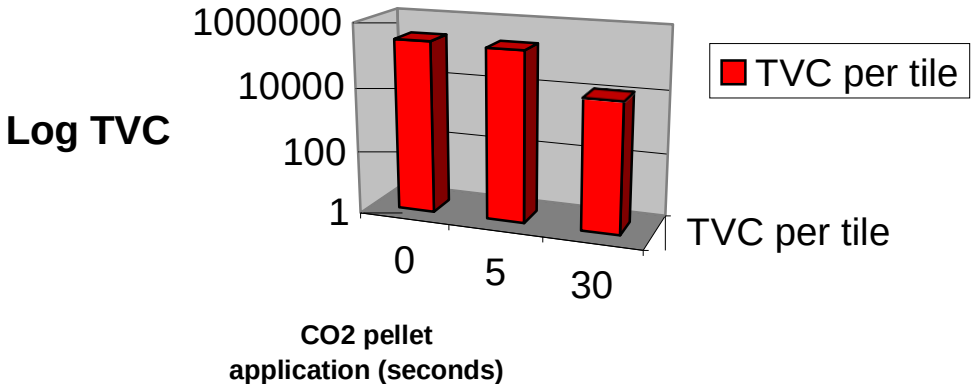
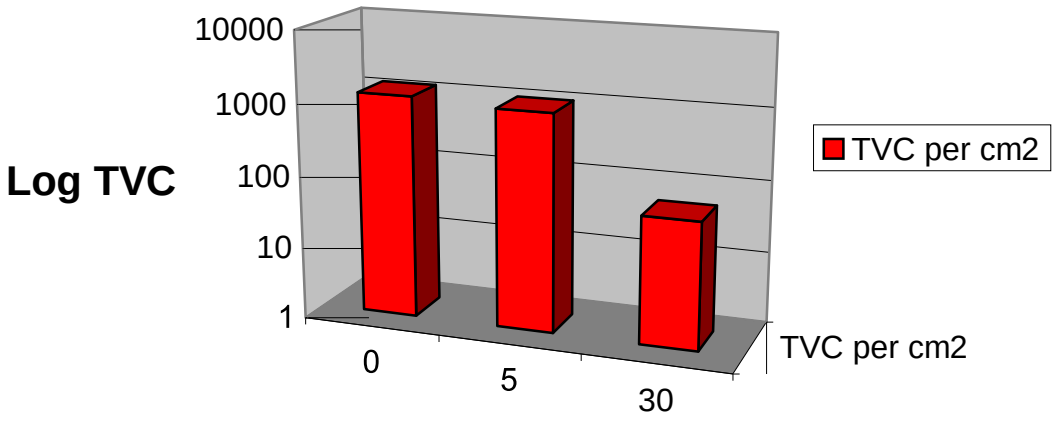


Figure 5.74 Effect of CO2 pellet application alone on quarry tiles on TVC S. enteritidis



The results for *E. coli* are shown in Figures 5.75 - 5.82 (Raw data are supplied in Appendix M) and show a 0.5 log reduction for ceramic tiles and a 3.0 log reduction for quarry tiles after 30 seconds treatment with CO_2 pellets alone. A reduction of only 0.5 log on ceramic tiles could be attributed to experimental error inherent in the method used for conducting TVC's, therefore care must be taken in interpreting the results for ceramic tiles. However, this compares with a 4.0 - 5.0 log reduction for ceramic tiles and a 2.0 log reduction with quarry tiles when using Cold Jet with CO_2 pellets (see Sections 5.4 & 5.5).

Figure 5.75 Effect of CO2 pellet application alone on ceramic tiles on TVC E. coli

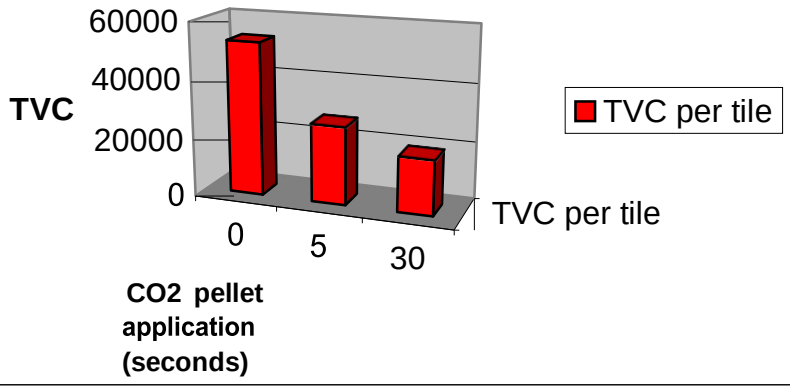


Figure 5.76 Effect of CO2 pellet application alone on ceramic tiles on TVC E. coli

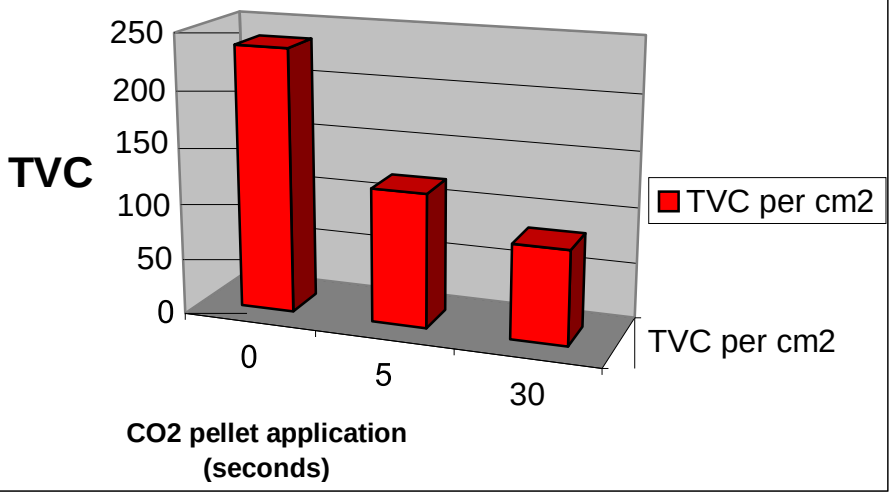


Figure 5.77 Effect of CO2 pellet application alone on ceramic tiles on TVC E. coli

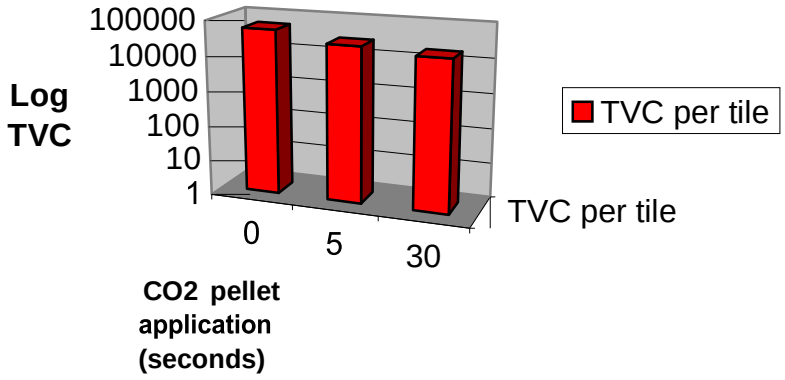


Figure 5.78 Effect of CO2 pellet application alone on ceramic tiles on TVC E. coli

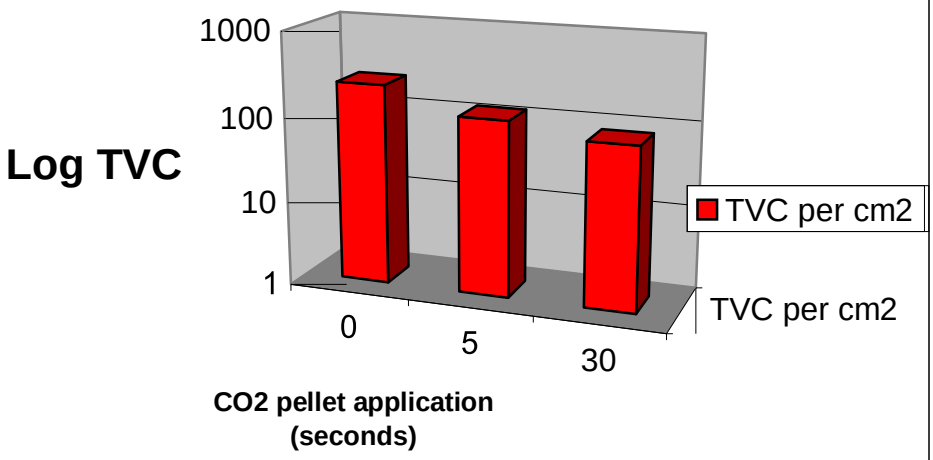


Figure 5.79 Effect of CO2 pellet application alone on quarry tiles on TVC E. coli

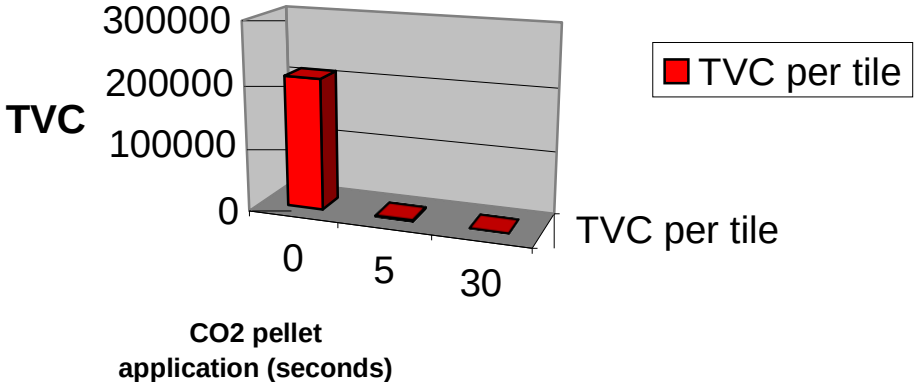


Figure 5.80 Effect of CO2 pellet application alone on quarry tiles on TVC E. coli

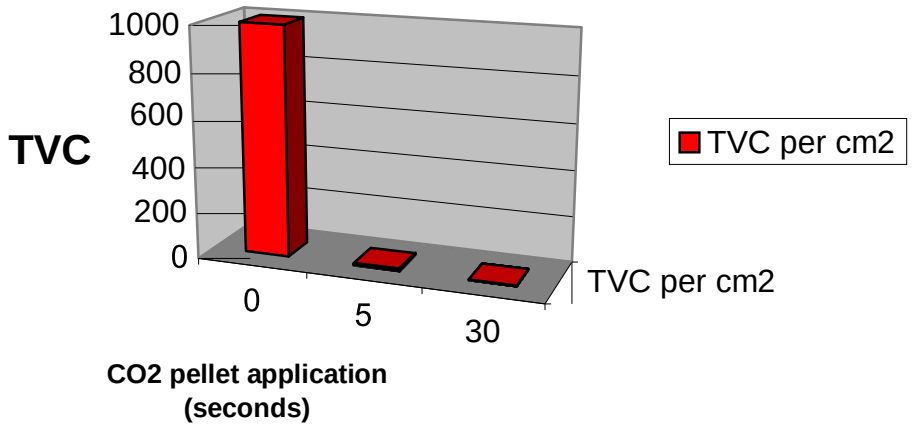


Figure 5.81 Effect of CO2 pellet application alone on quarry tiles on TVC E. coli

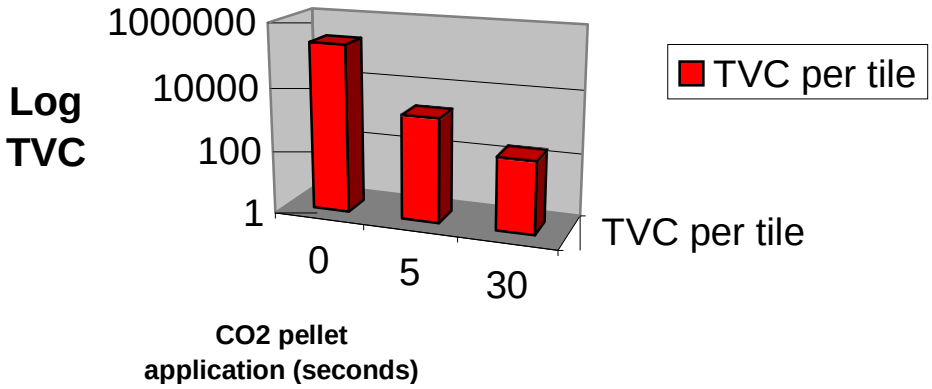
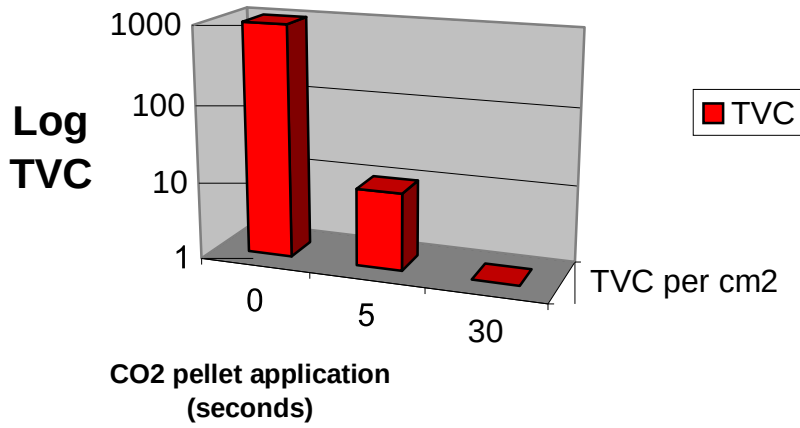


Figure 5.82 Effect of CO2 pellet application alone on quarry tiles on TVC E. coli



The results for *L. monocytogenes* are shown in Figures 5.83 - 5.90 (Raw data are supplied in Appendix M) and show a < 0.5 log reduction for ceramic tiles and a 2.0 log reduction for quarry tiles after 30 seconds treatment with CO_2 pellets alone. A reduction of only 0.5 log on ceramic tiles could be attributed to experimental error inherent in the method used for conducting TVC's, therefore care must be taken in interpreting the results for ceramic tiles. However, this compares with a 3.0 log reduction for ceramic tiles and a 3.0 log reduction with quarry tiles when using Cold Jet with CO_2 pellets (see Sections 5.6 & 5.7).

Figure 5.83 Effect of CO2 pellet application alone on ceramic tiles on TVC L. monocytogenes

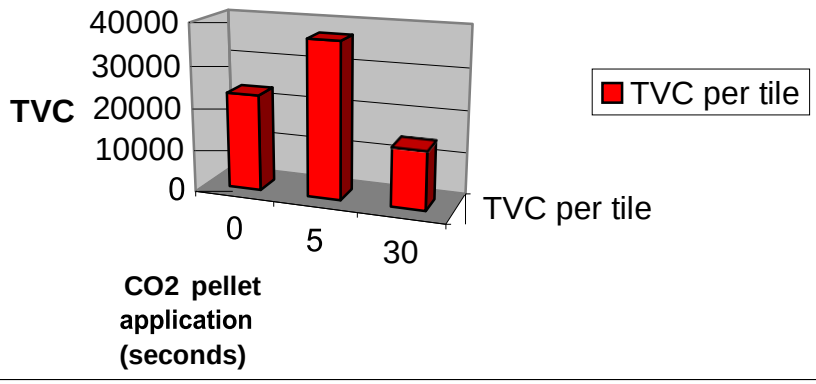


Figure 5.84 Effect of CO2 pellet application alone on ceramic tiles on TVC L. monocytogenes

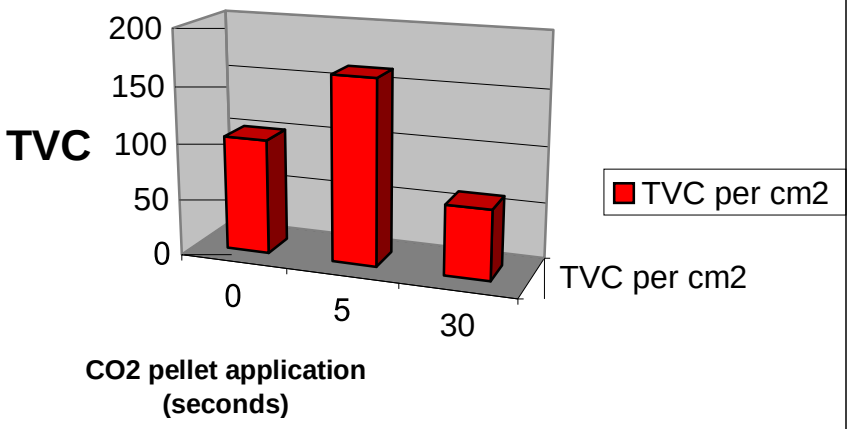


Figure 5.85 Effect of CO2 pellet application alone on ceramic tiles on TVC L. monocytogenes

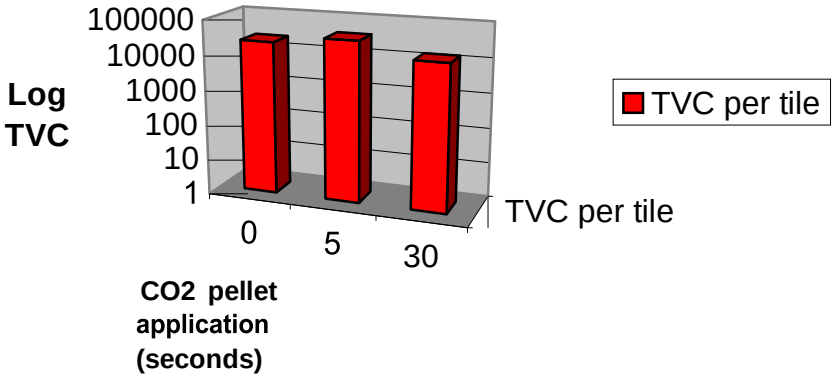


Figure 5.86 Effect of CO2 pellet application alone on ceramic tiles on TVC L. monocytogenes

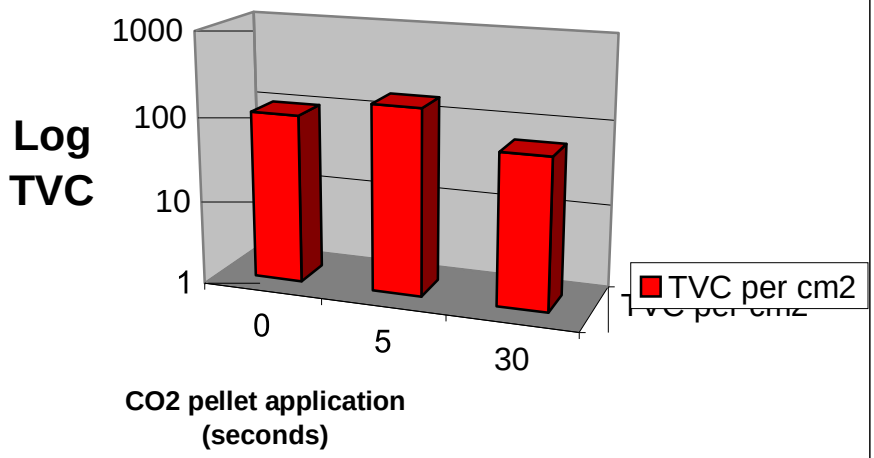


Figure 5.87 Effect of CO2 pellet application alone on quarry tiles on TVC L. monocytogenes

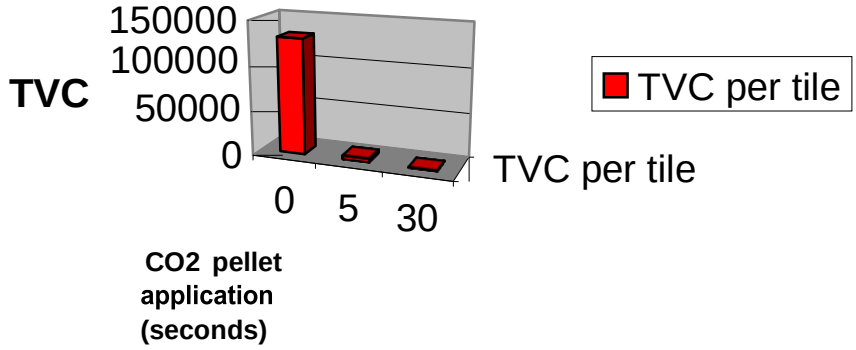


Figure 5.88 Effect of CO2 pellet application alone on quarry tiles on TVC L. monocytogenes

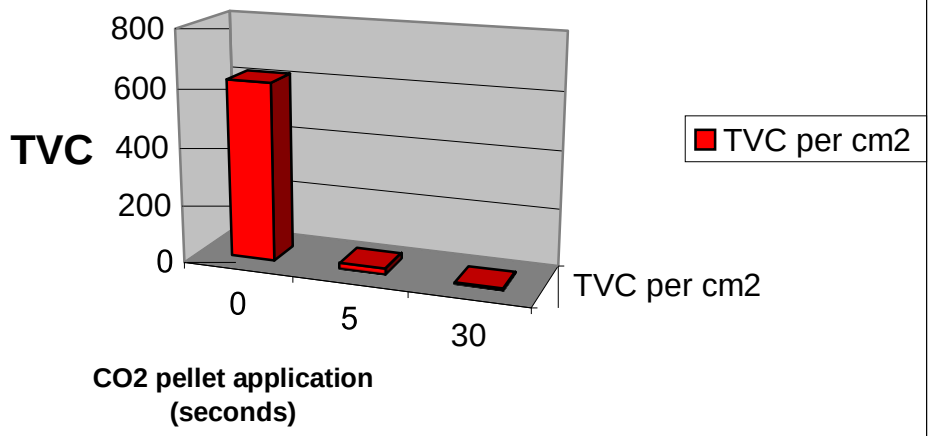


Figure 5.89 Effect of CO2 pellet application alone on quarry tiles on TVC L. monocytogenes

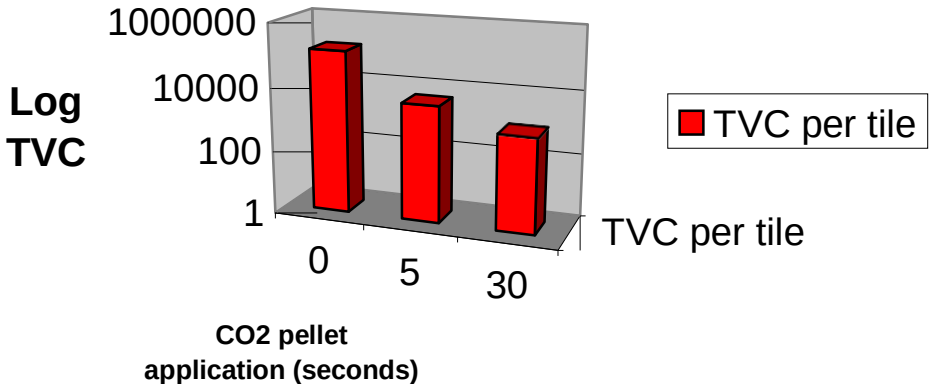
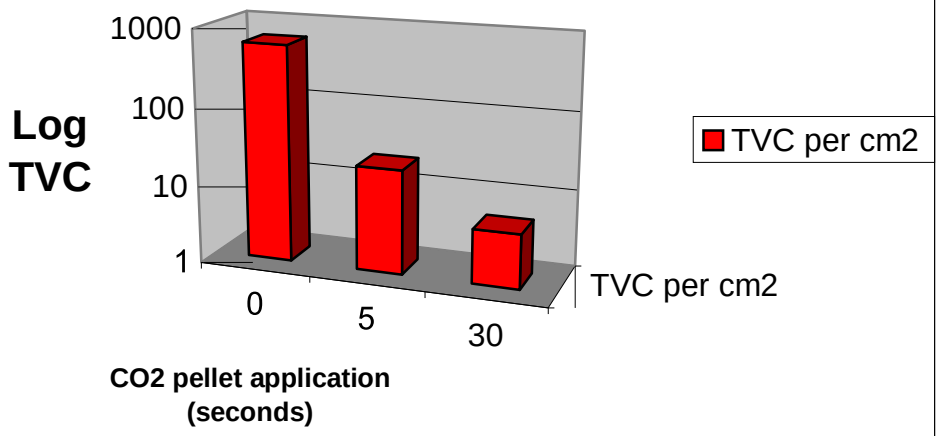


Figure 5.90 Effect of CO2 pellet application alone on quarry tiles on TVC L. monocytogenes



5.14 Use of Cold Jet in a fishmeal processing plant

The effectiveness of Cold Jet for the cleaning and decontamination of a fishmeal processing plant was evaluated. The Cold Jet system was used during the normal weekend cleaning and disinfection regime at the fishmeal plant. The fishmeal plant operates continuously from Sunday evening until Friday lunchtime, with a cleaning and disinfection schedule from Friday PM to Sunday AM. For these trials 3mm diameter CO_2 pellets and an outlet blast pressure of 50 psi and a pellet feeder rate of 30 were used. These parameters were decided on after preliminary work indicated that high blast pressure and high CO_2 pellet feeder rates were required to remove debris, and bearing in mind that the surfaces were stainless steel and did not contain any of the surfaces found to be susceptible to damage by Cold Jet, e.g. certain plastics, as found in Section 5.1.

The results are given in Figures 5.91 - 5.94 (Raw data are supplied in Appendix N). The results show a reduction in TVC's with some surfaces, e.g. swab 8 (rear screw), swab 10 (rear sampling point), and an increase in TVC's with other surfaces, e.g. swab 6 (screw next to main access hatch). Swabs 1- 10 were taken before any of the normal cleaning treatments were undertaken (the equipment in the line is normally hosed down, prior to spraying with disinfectant) thus there was a large amount of fishmeal blown about by the action of the Cold Jet and this likely led to re- contamination of areas after cleaning by Cold Jet. Thus swabs 3 and 6 show increased counts after Cold Jet treatment, whilst swabs 7 - 10 show the opposite.

Thus, further samples (swabs 11 - 20) were taken after the line had been hosed down (but not disinfected) and this again showed variable results, with swab 14 (bracket adjacent to access hatch) showing a 1 log reduction post-Cold Jet, whilst swab 17 (rear side groove/slot above screw) showed a 1.5 log increase post-Cold Jet. Swab 17 was taken from a narrow access panel into the process equipment and it was difficult to access properly with the Cold Jet system. Also, although reduced, there was still some fishmeal dust blown around during the operation of the Cold Jet after hosing. The effect of hosing generally resulted in much lower TVC's pre-Cold Jet application

than when no hosing was done (Appendix N), as hosing removed gross contamination, however in some cases TVC's pre-Cold Jet were increased by hosing (e.g. swab 17). The fishmeal processing lines are totally enclosed and are opened up for cleaning, thus the cleaning is taking place in confined spaces. Hence when using either Cold Jet or water hoses, the dirt and debris is blown around in confined spaces and thus debris often falls back onto surfaces that have already been cleaned. Therefore it is not surprising that the data in Appendix N is sometimes contradictory.

Swabs tested for *Salmonella* species were all negative, although some did show bacterial growth in the pre-enrichment BPWGL. However, growth in BPWGL was recorded less frequently from those swabs taken post Cold-Jet treatment than on those swabs taken pre-Cold Jet treatment (Table 5.2), with only 5 swabs giving growth post-Cold Jet compared with 13 swabs pre-Cold Jet. These differences were even more marked where the line was hosed down prior to Cold Jet treatment, with only 1 of swabs 11 - 20 showing growth post Cold Jet compared to 4 of swabs 1 - 10 showing growth post Cold Jet (Table 5.2).

Table 5.2 Growth in BPWGL pre-enrichment pre- and post-Cold Jet treatment

Swab No. (a, b)	Growth in BPWGL pre-enrichment pre-Cold Jet	Growth in BPWGL pre-enrichment (without hosing prior to Cold Jet)	Growth in BPWGL pre-enrichment (with hosing prior to Cold Jet)
1	-	-	
2	+	-	
3	+	+	
4	-	-	
5	+	+	
6	+	-	
7	-	-	
8	+	+	
9	+	+	
10	+	-	
11	-		-
12	-		-
13	+		-
14	+		+
15	-		-
16	+		-
17	+		-
18	+		-
19	-		-
20	+		-

(a) Swab Nos. 1 - 10 taken without hosing of the processing line prior to Cold Jet.

(b) Swab Nos. 11 - 20 taken after hosing of the processing line followed by Cold Jet.

Figure 5.91 TVC of various surfaces in a fishmeal plant pre- and post-Cold Jet

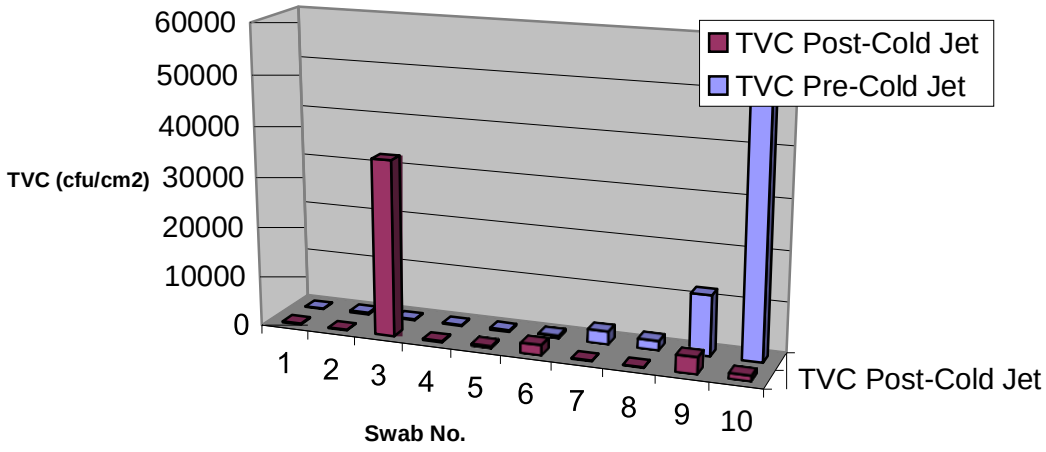


Figure 5.92 Log TVC of various surfaces in a fishmeal plant pre- and post-Cold Jet

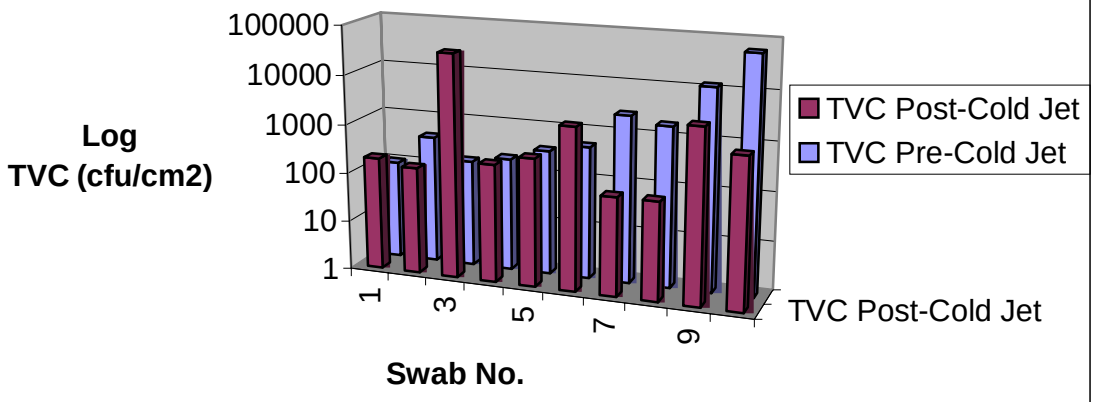


Figure 5.93 TVC of various surfaces in a fishmeal plant pre- and post-Cold Jet

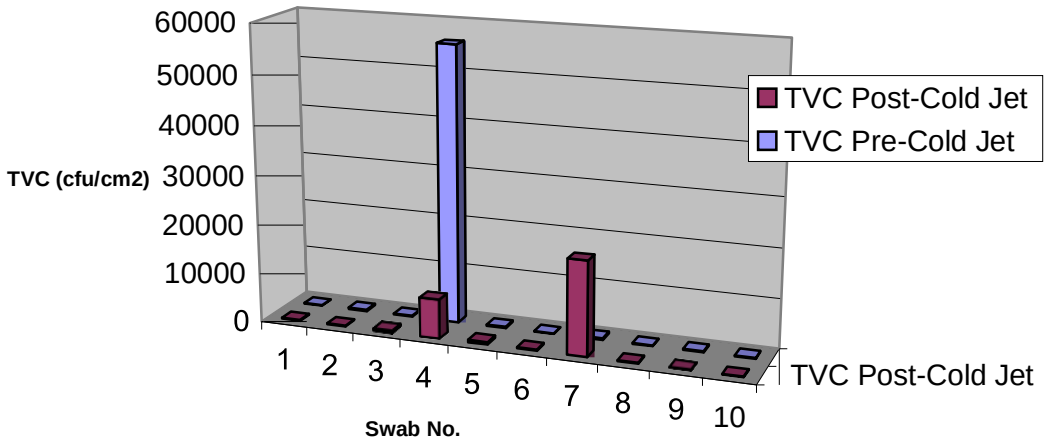
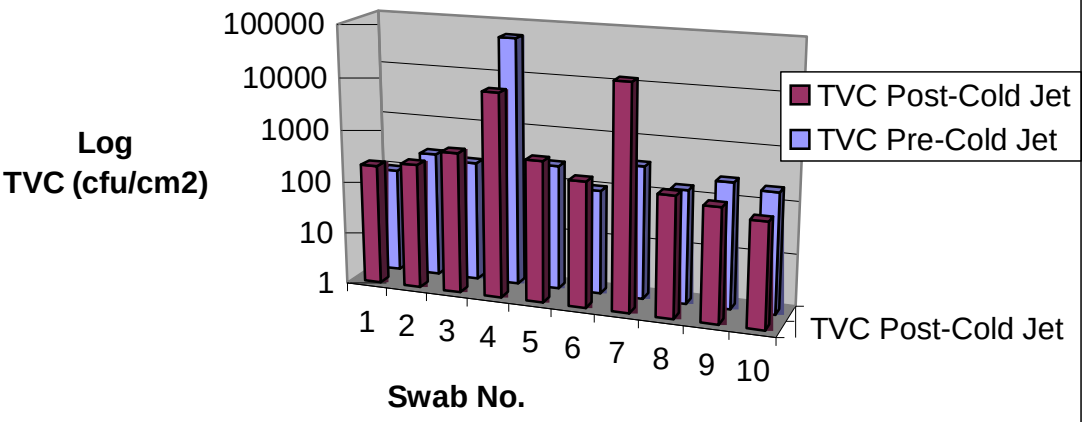


Figure 5.94 Log TVC of various surfaces in a fishmeal plant pre- and post-Cold Jet



5.15 Effect of treatment of meat portions and poultry carcasses with Cold Jet

To assess the effectiveness of using Cold Jet to decontaminate meat portions and poultry carcasses, meat and poultry portions were treated with 3mm diameter CO_2 pellets at an outlet blast pressure of 30 psi and a CO_2 pellet feeder rate of 30. Results for treating meat surfaces deliberately contaminated by application with spray cultures of *S. enteritidis*, *E. coli*, or *L. monocytogenes* are given in Tables 5.3 - 5.5 respectively, and results for treating poultry surfaces contaminated with *S. enteritidis*, *E. coli*, or *L. monocytogenes* are given in Tables 5.6 - 5.8 respectively.

Table 5.3 Effect of treatment of meat portions with Cold Jet on TVC *S. enteritidis*

Sample	TVC/ml	TVC/cm^2	TVC/g
Overnight culture	8.4×10^8		
Inoculum	7.7×10^6		
Time 0		1.0×10^4	
Post treatment (5 seconds)		2.2×10^1	
Debris			7.5×10^4

Table 5.4 Effect of treatment of meat portions with Cold Jet on TVC *E. coli*

Sample	TVC/ml	TVC/cm^2	TVC/g
Overnight culture	2.8×10^8		
Inoculum	2.3×10^6		
Time 0		1.2×10^4	
Post treatment (5 seconds)		1.8×10^1	
Debris			9.6×10^4

Table 5.5 Effect of treatment of meat portions with Cold Jet on TVC *L. monocytogenes*

Sample	TVC/ml	TVC/cm^2	TVC/g
Overnight culture	6.2×10^8		
Inoculum	3.8×10^6		
Time 0		1.1×10^4	
Post treatment (5 seconds)		1.3×10^1	
Debris			6.9×10^4

It can be seen from Table 5.3 that there was a 3 log reduction in *S. enteritidis* on meat after 5 seconds treatment with Cold Jet. Similar results were obtained for *E. coli* and *L. monocytogenes* (Tables 5.4 & 5.5). However, using the Cold Jet on meat portions resulted in significant damage to the meat tissue, with large parts of the meat blown off, and this residue was found to contain large numbers of *S. enteritidis* (Table 5.3), *E. coli* (Table 5.4), or *L. monocytogenes* (Table 5.5).

When treating whole chickens with Cold Jet, there was also a 3 log reduction in TVC's but again there was significant tissue damage with large amounts of poultry meat blown off the carcass (Tables 5.6 - 5.8), and high TVC's found in the poultry debris.

Table 5.6 Effect of treatment of whole chickens with Cold Jet on TVC *S. enteritidis*

Sample	TVC/ml	TVC/cm^2	TVC/g
Overnight culture	8.4×10^8		
Inoculum	7.7×10^6		
Time 0		3.6×10^4	
Post treatment (5 seconds)		4.7×10^1	
Debris			6.4×10^4

Table 5.7 Effect of treatment of whole chickens with Cold Jet on TVC *E. coli*

Sample	TVC/ml	TVC/cm^2	TVC/g
Overnight culture	2.8×10^8		
Inoculum	2.3×10^6		
Time 0		2.6×10^4	
Post treatment (5 seconds)		5.3×10^1	
Debris			4.0×10^4

Table 5.8 Effect of treatment of whole chickens with Cold Jet on TVC *L. monocytogenes*

Sample	TVC/ml	TVC/cm^2	TVC/g
Overnight culture	6.2×10^8		
Inoculum	3.8×10^6		
Time 0		1.9×10^4	
Post treatment (5 seconds)		2.4×10^1	
Debris			8.5×10^4

5.16 Determination of airborne contamination when using Cold Jet

In order to determine airborne contamination by *S. enteritidis, E. coli,* or *L. monocytogenes* seeded onto various surfaces when using Cold Jet, scatter plates were placed around the area where blasting was taking place. In addition, an air sampler was used to pump 750 Litres of air over single plates. Plates were exposed for the same length of time at which the Cold Jet was operated, e.g. 5 seconds, 30 seconds.

The results are given in Table 5.9 below. It can be seen that plate counts are higher when using the air sampler, which pumped a fixed volume of air over the plate compared with scatter plates which were subject only to air currents. In general, as the time of Cold Jet blasting increased, so the plate counts increased in all of the trials. As non-selective media were used (apart from XLD plates for *Salmonella*) these results are merely indicative of airborne contamination due to use of Cold Jet, but they do show that there is an increase in TVC per plate with increased time of Cold Jet application.

Table 5.9 Sampling for airborne contamination

Sample (a)	Surface	Cold Jet application time (seconds)	Scatter Plate (a)	Air Sampler Plate (a)
S. enteritidis	Plain white ceramic tile	5	4	130
S. enteritidis	Plain white ceramic tile	30	70	680
S. enteritidis	Plain white ceramic tile	5	1	10
S. enteritidis	Plain white ceramic tile	30	0	3
S. enteritidis	Plain red quarry tile	5	0	4
S. enteritidis	Plain red quarry tile	30	0	1
L. monocytogenes	Plain white ceramic tile	30	180	1890
L. monocytogenes	Plain red quarry tile	30	150	930
E. coli	Plain white ceramic tile	5	170	20
E. coli	Plain white ceramic tile	30	50	5
E. coli	Plain red quarry tile	5	30	70
E. coli	Plain red quarry tile	30	10	10
S. enteritidis	Plain white ceramic tile	10	135	1180
S. enteritidis	Plain white ceramic tile	30	530	1380
S. enteritidis	Plain red quarry tile	10	40	225
S. enteritidis	Plain red quarry tile	30	540	1440
L. monocytogenes	Plain white ceramic tile	5	20	280
L. monocytogenes	Plain white ceramic tile	30	50	310
L. monocytogenes	Plain red quarry tile	5	10	240
L. monocytogenes	Plain red quarry tile	30	40	400

(a) Counts are total TVC per plate.

6. Discussion:

6.1 Effectiveness of Cold Jet in decontaminating various food-grade surfaces

Cold Jet was shown to be very effective in reducing TVC's of *S. enteritidis* on surfaces made of stainless steel and various food grade plastics (see Section 5.1). In general, stainless steel showed larger log reductions (up to 5 log) in TVC *S. enteritidis* than food grade plastics, and this was probably due to a combination of factors in particular the smooth nature of the surface and it's imperviousness to penetration by liquids. In contrast, food grade plastics showed more varied results. Some, e.g. Acetal, showed a 3.0 log reduction in TVC *S. enteritidis* whilst a plastic conveyor belt showed only a 1.0 log reduction. There is a wide variation in the types and specifications of food grade plastics, thus some were able to withstand Cold Jet treatment, e.g. Acetal, PETP, whereas others were destroyed by Cold Jet treatment e.g. Sterisept. Also, in the case of the plastic conveyor belt this was a piece of a used conveyor belt, so the effects of wear and tear and the indented profile of its surface would assist in allowing bacteria to adhere and help to minimise contact of CO_2 pellets during Cold Jet treatment. However, it is clear that on smooth plastic surfaces that are resistant to the physical effects of Cold Jet treatment there is a marked reduction in TVC *S. enteritidis* (2.0 - 3.0 log) in most cases, with a few plastics only showing a 1.0 log reduction (e.g. PE500). Other plastics were either destroyed, e.g. Sterisept, or else irreversibly damaged, e.g. Vivak, by the Cold Jet treatment. Even with well used and heavily scratched polypropylene chopping boards, there was a 1.0 - 2.0 log reduction in TVC following Cold Jet treatment.

The cleaning and disinfection of food processing equipment and surfaces must be carried out regularly, but conventional methods only eliminate some microorganisms from equipment (Carpentier & Cerf, 1993), and there are problems with adherent microorganisms showing increased resistance to antimicrobials (Bloomfield *et al*, 1993). Also, all food processing surfaces have the potential to allow biofilm formation (Chamberlain & Johal, 1987), and formation of biofilms can occur even where hygiene and sanitation programmes are correctly applied (Notermans *et al*, 1991).

Cold Jet treatment has been shown to be effective in reducing populations of *Salmonella* on various types of surfaces used in food processing premises, and unlike chemical disinfectants there is no ability for the microorganisms to build up resistance to Cold Jet, and there are no chemical residues left behind that can taint the food. It is highly unlikely that biofilms could withstand Cold Jet treatment.

The very effective action of Cold Jet in removing large numbers of *Salmonella* from stainless steel is encouraging as stainless steel is the most frequently used material for food processing surfaces, and is as easily cleaned as glass, and more easily cleaned than plastic polymers, aluminium or copper (Boulange-Petermann, 1996). However, Cold Jet is also effective at cleaning and disinfecting several types of food grade plastics.

6.2 Effectiveness of Cold Jet in decontaminating plain white ceramic tiles

Cold Jet was found to be highly effective in decontaminating plain white ceramic tiles, of the type frequently used on walls in areas of food processing premises, for all three foodborne bacteria examined - *S. enteritidis, E. coli* and *L. monocytogenes* (see Sections 5.2, 5.4 & 5.6). There was a 3.0 - 4.0 log reduction in TVC for *S. enteritidis*, a 4.0 - 5.0 log reduction in *E. coli*, and a 3.0 log reduction in *L. monocytogenes* following treatment with Cold Jet. From these results it appears that Cold Jet has the greatest effect in reducing numbers of *E. coli*, followed by *S. enteritidis* and *L. monocytogenes*. It is probable that using Cold Jet to clean tiled surfaces would remove any loose grouting material, but as grouting should not be in a poor condition then this is not a major problem with using Cold Jet. Ideally smooth, impervious surfaces would be used for walls and so stainless steel or high impact resistant plastics would be suitable for decontamination with Cold Jet. Where wall tiles are used, then Cold Jet can be used for cleaning and disinfection provided the grouting is sound. Many modern food processing premises utilise PVC wall cladding and panels, and provided the plastic coatings are similar to some of the plastics tested here (e.g Acetal, PETP Ertalyte; Table 5.1) then it is reasonable to assume that Cold Jet would be suitable for decontaminating these surfaces. Painted surfaces found in food processing premises in areas away from direct contact with foods would probably not withstand Cold Jet

treatment unless the paint covering was sound and properly applied, as any damaged/cracked paint surfaces would allow Cold Jet to act to lift the paint and remove it (Cold Jet was originally designed to remove paint from aircraft).

As all types of food processing surfaces are potential sites for biofilm formation (Chamberlain & Johal, 1987), then the effectiveness of Cold Jet in cleaning and disinfecting plain ceramic tiles means that Cold Jet could be an effective system for use in cleaning these types of surfaces in food processing plants.

6.3 Effectiveness of Cold Jet in decontaminating quarry tiles

Cold Jet was found to be effective in decontaminating quarry tiles, of the type frequently used on floors in food processing premises, for all three foodborne bacteria examined - *S. enteritidis*, *E. coli* and *L. monocytogenes* (see Sections 5.3, 5.5 & 5.7). There was a 2.0 log reduction in TVC of *S. enteritidis*, a 2.0 log reduction in TVC *E. coli*, and a 3.0 log reduction in TVC *L. monocytogenes* after treatment with Cold Jet. Thus Cold Jet has the greatest effect on *E. coli*, followed by *L. monocytogenes* and *S. enteritidis*. For both plain ceramic tiles and quarry tiles, *E. coli* shows the greatest reduction in numbers, and this may be due to greater susceptibility to Cold Jet treatment compared to the other foodborne bacteria tested. As with ceramic tiles, any damaged grouting would be removed by the Cold Jet process, but again damaged grouting should be replaced and renewed in any food processing premises. Apart from
E. coli there was less of a log reduction in *S. enteritidis* and *L. monocytogenes* when compared with plain ceramic tiles. This may reflect the greater porosity of quarry tiles compared to ceramic tiles, as it was noted that after seeding quarry tiles with test microorganisms, the surface of these tiles dried out much more quickly than with ceramic tiles. However as with ceramic tiles, Cold Jet can be used for cleaning and disinfection of quarry tiles provided the grouting is sound.

As with plain ceramic tiles, quarry tiles could also be effectively cleaned and disinfected with Cold Jet, and would be a useful adjunct and/or replacement for conventional chemical disinfection methods (Bloomfield *et al*, 1993), and should also

prevent the formation of the types of biofilms found in food processing plants (Chamberlain & Johal, 1987).

6.4 Effect of varying CO_2 pellet feeder rate on Cold Jet treatment

S. enteritidis and *L. monocytogenes* were used as representative Gram negative and Gram positive foodborne bacteria for trials on the effect of varying the CO_2 pellet feeder rate (see Sections 5.8 - 5.11).

With the pellet feeder rate doubled from 30 to 60, for both plain ceramic tiles and quarry tiles the overall reduction in TVC *S. enteritidis* was approximately 2 log after 20 seconds treatment with Cold Jet, which is identical to that of treatment at half the pellet feeder rate. Similar levels of reduction in TVC *L. monocytogenes* were also found when the pellet feeder rate was doubled from 30 to 60. Therefore there is little point in increasing pellet feeder rate as this effectively means wasting CO_2 pellets as there is no beneficial increase in inactivation of bacteria when the CO_2 pellet feeder rate is increased. As CO_2 pellets are relatively expensive, it makes economic sense to use them as effectively as possible, therefore a lower pellet feeder rate is preferred. It was noticed, especially when doing prolonged Cold Jet blasting of 20 to 30 seconds that surfaces became very cold and that the CO_2 pellets did not always sublimate directly on impact but rather would 'stick' to the tiles and then sublimate off. Thus a low pellet feeder rate, and a low blast application time of 5 to 10 seconds would offer maximum effectiveness. This would also alleviate potential problems with airborne contamination (Section 6.9).

6.5 Effect of treating surfaces with air-blasting alone

The effect of air blasting alone on recovery of viable *S. enteritidis*, *E. coli* and *L. monocytogenes* was done to determine if any of the effects seen with Cold Jet were due to the action of air pressure alone removing bacteria from surfaces, and/or whether any drying effect of the air affected the viability of the test bacteria (see Section 5.12).

For *S. enteritidis* there was a 1.5 - 2.0 log reduction with air blasting compared to 2.0 - 4.0 log reduction when using Cold Jet, the corresponding figures for *E. coli* being 2.0 - 3.0 log and 4.0 - 5.0 log reductions respectively, and for *L. monocytogenes* 1.0 - 2.0 log and 3.0 log reductions respectively. Thus air blasting alone does reduce the TVC's of all three bacteria, but not to the same extent as that when using the entire Cold Jet system, i.e. air blasting with CO_2 pellets.

It is highly likely that most of the reduction in numbers of *S. enteritidis* and *L. monocytogenes* is due to the physical effects of air-blasting alone in removing cells that are loosely adhered to the surface, in the same way as rinsing with water would remove loosely attached microorganisms. These surfaces did not have biofilms present on them, and the test microorganisms were only presented to the surfaces for 8 minutes prior to treatment (Section 4.7) therefore there would have been insufficient time for these bacteria to establish attachment mechanisms. There may also have been a slight 'kill' effect by drying due to the flow of compressed air.

6.6 Effect of treatment of surfaces with CO_2 pellets alone

The effect of using CO_2 pellets alone on recovery of viable *S. enteritidis*, *E. coli* and *L. monocytogenes* was examined to see what the lethal effect exposing these bacteria to direct contact with CO_2 pellets at -78.5°C for up to 30 seconds would be (see Section 5.13).

With *S. enteritidis* there was a 1.5 log reduction when using CO_2 pellets alone compared to 2.0 - 4.0 log reduction when using Cold Jet. *E. coli* showed corresponding figures of 0.5 - 3.0 log and 4.0 - 5.0 log reductions respectively, and *L. monocytogenes* 0.5 - 2.0 log and 3.0 log reductions respectively. Thus all three bacteria show reductions in TVC's when exposed to CO_2 pellets alone, but not to the same extent as that when using the entire Cold Jet system i.e. with air blasting too.

6.7 Use of Cold Jet in a fishmeal processing plant

Cold Jet was evaluated in a fishmeal processing premises, as fishmeal is used as an animal feed (and is a potential source of *Salmonella* and other Enterobacteriaceae), and is also an example of a difficult type of food processing premises to clean and disinfect due to the dry and fibrous nature of fishmeal. Animal feeds are frequently contaminated with *Salmonella* which can then be transmitted through the food chain and lead to eventual foodborne illness (Crump *et al* 2002). Results were mixed for the first batch of swabs (1- 10; see Section 5.14), mainly due to the Cold jet moving dust and gross debris around the fishmeal plant, with increases in TVC's for 2 and decreases in TVC's for 4 of the areas tested. These areas had a large amount of fishmeal present and would normally be washed by hosing prior to the main cleaning and disinfection regime. This was not done prior to Cold Jet treatment in order to ascertain the efficiency of Cold Jet for this purpose. When using the Cold Jet there were difficulties in actually seeing the area being cleaned due to the movement of dirt and other debris by the Cold Jet. As the results showed, some areas were being recontaminated after Cold Jet treatment due to the movement of this debris.

Therefore, this was repeated using the Cold Jet after the initial hosing down of the processing plant and this gave better results. There were still some swabs (from swabs 11 - 20) that gave an increase in TVC post Cold Jet, but this was due to the nature of the processing plant, with some areas chosen for testing the Cold Jet as they had difficult access points and so it was difficult to apply the Cold Jet directly to these areas.

Swabs were also taken of the areas pre- and post-Cold Jet treatment and these showed that with Cold Jet used without prior hosing of the processing plant, 7 out of 10 swabs showed growth pre-Cold Jet and 4 out of 10 swabs showed growth post-Cold Jet. When Cold Jet was used after hosing the processing plant, 6 out of 10 swabs showed growth pre-Cold Jet and 1 out of 10 swabs showed growth post-Cold Jet. Thus there was a dramatic decrease in microbiological contamination when Cold Jet was used to clean and disinfect after an initial hosing down of the processing plant. This again shows the effectiveness of using Cold Jet to decontaminate stainless steel, especially given that the use of enrichment media to recover viable bacteria from the swabs means that in effect the growth observed could be of one single cell recovered by the

swab (see Section 6.1). As the Cold Jet system used here was a free standing unit with no special modifications or adaptations to allow its use in this processing plant, there is certainly scope for modifying and integrating Cold Jet into the process line, e.g. in the driers. This would allow the cleaning and disinfection of the process line to be undertaken both as a normal weekly cycle, but also would allow cleaning and disinfection to take place at regular intervals or, where practical, even continuously.

6.8 Treatment of meat portions and poultry carcasses with Cold Jet

Using Cold Jet to decontaminate meat portions and poultry carcasses, it was able to significantly reduce the level of contamination with *S. enteritidis*, *E. coli* and *L. monocytogenes* by up to 10^3 cfu/cm^2 (see Section 5.15). However, there was significant damage to the meat and poultry tissues, with large fragments of tissue detached from the main body. In particular, the meat portions suffered a large amount of damage and were not in a saleable condition following Cold Jet treatment. Also, high numbers of *S. enteritidis*, *E. coli* and *L. monocytogenes* were found in the tissue fragments recovered after Cold Jet treatment.

Various methods for decontaminating meat and chicken carcasses have been used including chemical methods such as lactic acid (Gill & Badoni, 2004; van der Marel *et al*, 1988, Sakhare *et al* 1999), hydrogen peroxide (Lillard & Thomson, 1983; Wagenaar & Snijders, 2004), sodium triphosphate (Whyte *et al*, 2001), peroxyacetic acid (Gill & Badoni, 2004) and chlorine (Gill & Badoni, 2004; James *et al*, 1992; Whyte *et al*, 2001). In addition ionising radiation has been advocated for reducing the microbial pathogen loading on meat and poultry carcasses (Mossel & Stegeman, 1985). Chemical methods have many disadvantages including ensuring that the correct concentration is used, allowing sufficient contact time, rinsing/removal of residues after treatment and recontamination after rinsing. Ionising radiation is effective in removing microbial contamination from foods, but requires expensive specialist plant which is not usually conveniently located for food processors, but above all faces fierce consumer resistance.

Despite the problems found with these trials with Cold Jet for decontaminating meat and poultry, there is potential for Cold Jet to be used in slaughtering and dressing of carcasses as unlike chemical methods it is relatively straightforward to set up and use, with no dilution/mixing required, no liquid waste to dispose of, and no possibility of taint residues being left. For Cold Jet to be used to decontaminate meat and poultry immediately after slaughter and dressing, the Cold Jet system would have to be modified, preferably to blast pellets at a lower rate, and in particular with a significant decrease in the volume of compressed air passed through the blasting nozzle.

6.9 Airborne contamination when using Cold Jet

Microorganisms were found in the atmosphere adjacent to where Cold Jet blasting was undertaken at increasing levels with increasing Cold Jet application time (see Section 5.16). This is not altogether surprising, as the initial inocula used in the trials were very large, so the test surfaces being examined probably had a higher level of contamination than would normally be found in a food processing environment, especially that for *Salmonella*, and the high flow rates of compressed air used would enhance the dispersal of these bacteria into the atmosphere. Also as most surfaces were only allowed to dry for 3 minutes following dipping in liquid cultures then there was insufficient time to allow the bacteria to adhere to the surfaces. In a 'real life' situation, these bacteria would probably be encountered as part of a biofilm (Chamberlain & Johal, 1987), especially where cleaning and disinfection schedules were not adhered to properly, or where parts of food processing machinery were not easily accessed for cleaning and disinfection (Carpentier & Cerf, 1993; Notermans *et al*, 1991). As it has been shown that the effect of CO_2 pellets is lethal to these bacteria on certain surfaces (see Section 6.6), then it is probable that the combined action of Cold Jet - the kinetic effect on impact of the CO_2 pellets, the direct sublimation from solid to gas of the CO_2 pellets, the thermal shock of the CO_2 pellets at -78.5°C, and the lifting off of debris by the blasting of compressed air - then all of these factors combine to give a lethal effect. However, due to the large numbers of bacteria used in the inocula and the fact that they were not part of an established biofilm then some would survive to be recovered on the scatter and air sampler plates after dispersal in the compressed air stream.

Food processing equipment and premises can be microbiologically contaminated from environmental routes such as air currents, humans, animals, insects coming in to contact with surfaces. For food product contamination, the most important source of contamination is via direct contact with contaminated surfaces (Holah, 1995). Cold Jet has been shown to be effective in reducing surface contamination. Although large numbers of microorganisms are inactivated by Cold Jet, because of the large inocula used here there is an aerosol created on use. This aerosol could be reduced by pre-cleaning, e.g. with water, prior to Cold Jet application. Also, as the level of contamination found in food processing environments would be very much less, aerosols would not be so highly contaminated. In addition, it is common practice to use high pressure hoses in food processing environments, and these have no bactericidal effect and create aerosols. The Cold Jet system would represent a significant improvement on this as it does have a bactericidal effect. The Cold Jet system could also be redesigned to form an integral part of a food processing line within an enclosed system, thus minimising aerosols. Areas where Cold Jet could be used include food processing equipment (poultry defeathering machines, eviscerators, carcass washing machines, refrigeration and freezing equipment, cookers and driers), walls (tiled, PVC clad), floors (tiled), food preparation areas (stainless steel tables, sinks, protective guards, etc.).

Acknowledgements:

Microchem Bioscience Limited gratefully acknowledges the support and funding of this project by the Food Standards Agency, Microbiological Safety Division, Aviation House, 125 Kingsway, London, WC2B 6NH. Microchem Bioscience Limited also thanks the assistance of many of the Food Standards Agency staff including Dr Jonathan Back, Mrs Marion Castle and Mrs June Lock.

The technical help and support of the Food Standards Agency Programme Manager for this project, Mr Mike Jacob, is also gratefully acknowledged.

The technical assistance and help of Ms Alison Watt and Dr Mark Brown of Microchem Bioscience Limited is gratefully acknowledged.

Many thanks are due to Dr Dan Lloyd of Cold Jet (Europe) Limited for his help and technical support during this project.

The assistance of the staff at United Fish Products Limited during the cleaning trials is gratefully acknowledged, in particular Mr Helge Korsager, Ms Alison Chree, Mr Marshall Harper and Mr Ian Mitchell.

The provision of surface test materials (stainless steel, plastic, etc.) by Alex Ross Limited, ABG Rubber and Plastics Limited, Norfo A/S and Liquid Plastics Limited is gratefully acknowledged.

The provision of technical assistance by Dr David Gray of the Food Science & Technology Research Centre is gratefully acknowledged.

Microchem Bioscience Limited would also like to thank and acknowledge the help and support of the many other individuals and organisations that provided help and

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Appendices

Appendix A: Raw Data for Results 5.1.

Appendix A: Results of Cold Jet treatment of various surfaces							
Surface	Length (cm)	Width (cm)	Area (cm2)	TVC Pre-Cold Jet	TVC/cm 2	TVC Post-Cold Jet	TVC/cm 2
1	12	5.5	66	500000	15152	13000	394
2	12	7	84	640000	15238	3800	90
3	8	5	40	3700000	185000	580	29
4	19.5	10	195	2300000	23590	120000	1231
5	12	10	120	8400000	140000	300	5
6	10	10	100	460000	9200	2700	54
7	10	10	100	300000	6000	20000	400
8	10	10	100	320000	6400	400	8
9	10	10	100	310000	6200	1000	20
10	10	10	100	740000	14800	800	16
11	13.5	10.5	141.75	2400000	33862	700	10
12	10	10	100	130000	2600	0	0
13	12	14	168	4700000	55952	300	4
14	10	8	80	6500000	162500	3700	93
15	8	7	56	8200000	292857	240000	8571
16	5	2	10	6700000	1340000	270	54
17	8	2	16	4300000	537500	440	55
18	12	7.5	90				0
19	12	7.5	90	900000	20000	110	2
20	12	7.5	90	870000	19333	0	0
21	12	7.5	90	1200000	26667	2200	49
22	12	7.5	90	1900000	42222	6300	140
23	12	7.5	90				0
24	10	12	120				0
25	12	14	168				0

Appendix B: Raw Data for Results 5.2

Appendix B: Raw Data for Results 5.2 Salmonella enteritidis								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	4100000	18222		0	4100000		0	18222
5	52000	231		5	52000		5	231
10	17000	76		10	17000		10	76
15	17000	76		15	17000		15	76
20	1900	8		20	1900		20	8
30	500	2		30	500		30	2

Appendix C: Raw Data for Results 5.3

Appendix C: Raw Data for Results 5.3 Salmonella enteritidis								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	1000000	4756		0	1000000		0	4756
5	110000	523		5	110000		5	523
10	3400	16		10	3400		10	16
15	5200	25		15	5200		15	25
20	2000	10		20	2000		20	10
30	17000	81		30	17000		30	81

Appendix D: Raw Data for Results 5.4

Appendix D: Raw Data for Results 5.4 E. coli								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	850000	3778		0	850000		0	3778
5	3100	14		5	3100		5	14
10	300	1		10	300		10	1
15	0	0		15	1		15	1
20	0	1		20	1		20	1
30	0	0		30	1		30	1

Appendix E: Raw Data for Results 5.5

Appendix E: Raw Data for Results 5.5 E. coli								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	97000	461		0	97000		0	461
5	700	3		5	700		5	3
10	200	1		10	200		10	1
15	0	0		15	1		15	1
20	0	0		20	1		20	1
30	200	1		30	200		30	1

Appendix F: Raw Data for Results 5.6

Appendix F: Raw Data for Results 5.6 Listeria monocytogenes								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	210000	933		0	210000		0	933
5	5900	26		5	5900		5	26
10	2500	11		10	2500		10	11
15	600	3		15	600		15	3
20	4400	20		20	4400		20	20
30	300	1		30	300		30	1

Appendix G: Raw Data for Results 5.7

Appendix G: Raw Data for Results 5.7 Listeria monocytogenes								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	190000	904		0	190000		0	904
5	7800	37		5	7800		5	37
10	3400	16		10	3400		10	16
15	800	4		15	800		15	4
20	700	3		20	700		20	3
30	600	3		30	600		30	3

Appendix H: Raw Data for Results 5.8

Appendix H: Raw Data for Results 5.8 Salmonella enteritidis								
Plain White Ceramic Tiles								
Pellet Feeder Rate = 60								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	730000	3244		0	730000		0	3244
5	1100	5		5	1100		5	5
10	1700	8		10	1700		10	8
20	2500	11		20	2500		20	11

Appendix I: Raw Data for Results 5.9

Appendix I: Raw Data for Results 5.9 Salmonella enteritidis								
Plain Red Quarry Tiles								
Pellet Feeder Rate = 60								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	1300000	6183		0	1300000		0	6183
5	7200	34		5	7200		5	34
10	8800	42		10	8800		10	42
20	1700	8		20	1700		20	8

Appendix J: Raw Data for Results 5.10

Appendix J: Raw Data for Results 5.10 Listeria monocytogenes								
Plain White Ceramic Tiles								
Pellet Feeder Rate = 60								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	96000	427		0	96000		0	427
5	100	0		5	100		5	1
10	200	1		10	200		10	1
20	200	1		20	200		20	1

Appendix K: Raw Data for Results 5.11

Appendix K: Raw Data for Results 5.11 Listeria monocytogenes								
Plain Red Quarry Tiles								
Pellet Feeder Rate = 60								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	110000	523		0	110000		0	523
5	1000	5		5	1000		5	5
10	300	1		10	300		10	1
20	300	1		20	300		20	1

Appendix L: Raw Data for Results 5.12

Appendix L: Raw Data for Results 5.12 Salmonella enteritidis								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	4100000	18222		0	4100000		0	18222
5	470000	2089		5	470000		5	2089
30	73000	324		30	73000		30	324
Appendix L: Raw Data for Results 5.12 Salmonella enteritidis								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	1000000	4756		0	1000000		0	4756
5	260000	1237		5	260000		5	1337
30	64000	304		30	64000		30	304
Appendix L: Raw Data for Results 5.12 E. coli								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	2500000	11111		0	2500000		0	11111
5	260000	1156		5	260000		5	1156
30	21000	93		30	21000		30	93
Appendix L: Raw Data for Results 5.12 E. coli								
Plain Red Quarry Tiles								

Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	190000	904		0	2500000		0	11891
5	86000	409		5	43000		5	205
30	19000	90		30	7300		30	35
Appendix L: Raw Data for Results 5.12 Listeria monocytogenes								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	210000	933		0	210000		0	933
5	140000	622		5	140000		5	622
30	3100	14		30	3100		30	14
Appendix L: Raw Data for Results 5.12 Listeria monocytogenes								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	190000	904		0	190000		0	904
5	86000	409		5	86000		5	409
30	19000	90		30	19000		30	90

Appendix M: Raw Data for Results 5.13

Appendix M: Raw Data for Results 5.13 Salmonella enteritidis								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	910000	4044		0	910000		0	4044
5	700000	3111		5	700000		5	3111
30	60000	267		30	60000		30	267
Appendix M: Raw Data for Results 5.13 Salmonella enteritidis								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	260000	1237		0	260000		0	1237
5	220000	1046		5	220000		5	1046
30	12000	57		30	12000		30	57
Appendix M: Raw Data for Results 5.13 E. coli								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	53000	236		0	53000		0	236
5	27000	120		5	27000		5	120
30	19000	84		30	19000		30	84
Appendix M: Raw Data for Results 5.13 E. coli								
Plain Red Quarry Tiles								

Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	210000	999		0	210000		0	999
5	2000	10		5	2000		5	10
30	200	1		30	200		30	1
Appendix M: Raw Data for Results 5.13 Listeria monocytogenes								
Plain White Ceramic Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	23000	102		0	23000		0	102
5	37000	164		5	37000		5	164
30	14000	62		30	14000		30	62
Appendix M: Raw Data for Results 5.13 Listeria monocytogenes								
Plain Red Quarry Tiles								
Application Time	TVC	TVC/cm2		Application Time	TVC		Application Time	TVC/cm2
0	130000	618		0	130000		0	618
5	4600	22		5	4600		5	22
30	1000	5		30	1000		30	5

Appendix N: Raw Data for Results 5.14

Appendix N: Raw Data for Results 5.14		
Swab No. (*)	TVC (cfu/cm2) Pre- Cold Jet	TVC (cfu/cm2) Post- Cold Jet
1	100	200
2	400	150
3	150	35000
4	200	250
5	350	400
6	500	2000
7	2500	100
8	1800	100
9	12000	3300
10	60000	1100
11	100	200
12	250	250
13	200	500
14	56000	7900
15	250	500
16	100	250
17	350	19000
18	150	200
19	250	150
20	200	100

(*) Swab nos. 1 - 10 were taken without hosing of the processing line (with cold water) prior to Cold Jet application. Swab nos. 11 - 20 were taken after hosing of the processing line (with cold water) prior to Cold Jet application.