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DISINFECTION OF SOLID SURFACES BY COMBINATIONS OF UV LIGHT AND OXIDATION TREATMENTS

by

Douglas Walter McGregor Gardner

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of

Doctor of Philosophy of Loughborough University

March 1997

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Combination of ultraviolet light (UV) and oxidising agents, hydrogen peroxide (H₂O₂) and ozone (O₃), were evaluated for disinfecting solid surfaces. Studies were conducted using spores of the bacterium *Bacillus subtilis* (ATCC 6633) attached to materials ranging from filter papers and membranes to aluminium and PTFE. Techniques were developed for depositing, recovering and enumerating spores from these surfaces.

Treatment of spore-laden surfaces was carried out both with bench-top mounted UV sources and in a specially designed irradiation chamber. Objects for treatment in the latter were suspended in the UV field created by four symmetrically arranged sources. The chamber was equipped with an air-atomising nozzle so that objects could be treated with sprays of aqueous oxidants whilst undergoing irradiation. Provision was also made for electrostatically charging the nozzle.

Kinetic data was collected on the inactivation of spores by various combinations of UV and oxidising agents. Synergistic inactivation of spores was observed for UV and H₂O₂, with a particularly high rate of inactivation being recorded for H₂O₂ concentrations in the range 0.5 - 1.0 % w/v H₂O₂. Aqueous ozone did not exhibit sporicidal action under the conditions investigated. Some evidence was obtained to show that electrostatic charging of sprays did result in enhanced rates of surface impact, but the results obtained were inconclusive.

The structure of the surface on which spores were present was shown to influence the disinfection levels obtained when the surface was exposed to UV. A zonal shielding model was developed in which the surface was assumed to comprise of a finite number of zones in which the spores were afforded specific protection to incident UV. This model was fitted to the experimental data with good results.

Modelling of the UV intensity fields created by UV sources and the intensities produced on the surfaces of objects within UV fields was conducted. These models were combined with experimentally-derived kinetic expressions to predict the disinfection rates on objects of regular geometry undergoing treatment either with UV or with UV in combination with H₂O₂.
ACKNOWLEDGEMENTS

I wish to thank EA Technology for their funding of this project. Many thanks to Dr. Peter Francis for giving me the opportunity to undertake this research program and to Dr. Jim Hutchison for his continued support over the past four years.

Many thanks to my supervisor, Dr. Gilbert Shama, for his guidance during the research for this project and the writing of this thesis.

The work contained within this thesis would not have been possible without the able assistance of the technical staff in the Chemical Engineering department. Many thanks to David Smith for his help and advice in the microbiology laboratory, to Hugh Peters for his advice in the construction and design of the spray disinfection chamber, to Terry Neale for the UV lamp system and the electrostatic spraying system. The guys in the workshop were very helpful during this research and I would wish to thank Tony, Barry, Pip and Leo for all their help and assistance in constructing the spray disinfection chamber and the test objects used within it.

I would like to thank Dr. Ian Stenhouse for his invaluable advice about sprays. Thanks also to Ian Sinclair for his advice on electrostatic spraying. The scanning electron microscope pictures in this thesis were produced by Frank Page.

Many thanks to Carole Reid and Chris Peppiatt, my colleagues in the laboratory, for their helpful advice regarding micro-organisms and microbial lab techniques. Thanks also to Chris for scanning the electron microscope pictures for this thesis.

I would also wish to thank my parents for their support during this study.
# TABLE OF CONTENTS

## 1. INTRODUCTION

1.1 Surface disinfection
   - 1.1.1 Definition of terms...
   - 1.1.2 Scope of application...
   - 1.1.3 Disinfectants...

1.2 UV irradiation
   - 1.2.1 Introduction...
   - 1.2.2 Disinfection with UV...
   - 1.2.3 Disinfection kinetics...
   - 1.2.4 Mechanism of UV-induced inactivation...
   - 1.2.5 Repair and recovery of UV-induced damage...
     - 1.2.5.1 Repair mechanisms for UV-induced damage...
     - 1.2.5.2 Photoreactivation and Photoenzymatic repair...
     - 1.2.5.3 Excision - Resynthesis repair...
     - 1.2.5.4 Recombination repair...
     - 1.2.5.5 Repair mechanisms for damage caused by other sterilants...

1.3 Peroxidation
   - 1.3.1 Introduction...
   - 1.3.2 Disinfection using hydrogen peroxide...
   - 1.3.3 Mechanisms of microbial inactivation...

1.4 Ozonation
   - 1.4.1 Introduction...
   - 1.4.2 Disinfection using ozone...
   - 1.4.3 Mechanism of disinfection...
1.5 Combined treatments (Advanced oxidation methods)
   1.5.1 Introduction
   1.5.2 UV and hydrogen peroxide
      1.5.2.1 Disinfection
      1.5.2.2 Mechanisms of microbial inactivation and hydroxyl radical formation
   1.5.3 Ozone and hydrogen peroxide
      1.5.3.1 Disinfection using aqueous O\textsubscript{3} and H\textsubscript{2}O\textsubscript{2}
      1.5.3.2 Mechanisms of inactivation and hydroxyl radical formation
   1.5.4 UV and ozone
      1.5.4.1 Introduction
      1.5.4.2 Mechanisms of hydroxyl radical generation

1.6 Aims and Objectives

2. METHODS AND APPARATUS

2.1 Micro-organisms
   2.1.1 Source
   2.1.2 Cultivation of organisms
   2.1.3 \textit{B.subtilis} spore production
   2.1.4 Determination of UV resistance of spores

2.2 Coating of surfaces
   2.2.1 Introduction
   2.2.2 Filter paper strips
   2.2.3 Membranes
   2.2.4 Non-porous materials
   2.2.5 Attachment to objects
2.3 Lab-based inactivation studies
   2.3.1 Introduction 42
   2.3.2 Exposure to UV 42
   2.3.3 Exposure to liquid sterilants 42
   2.3.4 Exposure to gaseous ozone 45

2.4 Assessment of microbial survival following disinfection
   2.4.1 Serial dilutions 45
   2.4.2 Plating 45
   2.4.3 Sampling precautions 46

2.5 Chamber description 46 - 56

2.6 Inactivation studies in the chamber
   2.6.1 Exposure to UV 56
   2.6.2 Exposure to sterilant sprays 56 - 58
   2.6.3 UV bioassay 58

2.7 Spray impact tests
   2.7.1 Introduction 60
   2.7.2 Absorbance method 60
   2.7.3 Attached filter paper method 60

2.8 Production and measurement of ozone
   2.8.1 Production of ozone 61
   2.8.2 Measurement of gaseous ozone concentration 63
   2.8.3 Measurement of aqueous ozone concentration 63

3. DISINFECTION EXPERIMENTS

3.1 Introduction 64
   3.1.1 Inactivation kinetics 64 - 66
3.2 UV irradiation
   3.2.1 Results
   3.2.2 Discussion

3.3 Disinfection using hydrogen peroxide
   3.3.1 Introduction
   3.3.2 Results
   3.3.3 Discussion

3.4 Combined UV and H₂O₂ disinfection
   3.4.1 Introduction
   3.4.2 Results
   3.4.3 Discussion

3.5 Disinfection using ozone and ozone combined with UV
   3.5.1 Introduction
   3.5.2 Results
   3.5.3 Discussion

3.6 Disinfection experiments employing electrostatically-charged sprays
   3.6.1 Introduction
   3.6.2 Results
   3.6.3 Discussion

3.7 Overall discussion and conclusions

4. MATHEMATICAL MODELLING OF SURFACE SHIELDING
   4.1 Introduction
   4.2 Mathematical description
   4.3 Solving the model
4.4 Results 118 - 123
4.5 Discussion 123 - 126
4.6 Conclusions 127
4.7 Notation 128

5. UV FIELD MODELLING
5.1 Introduction 129 - 131
5.2 Mathematical description 131 - 134
5.3 Verifying the mathematical models 134 - 136
5.4 Predicting UV intensity fields on surfaces 136
5.4.1 Cylindrical geometry 138 - 145
5.4.2 Slab geometry 145 - 148
5.5 Conclusions 148
5.6 Notation 149

6. COMBINED UV-H₂O₂ DISINFECTION MODEL
6.1 Introduction 150
6.2 Model description 150 - 153
6.2.1 General equations 153 - 158
6.2.2 Example 153 - 158
6.3 Results 159 - 165
6.4 Discussion 165 - 171
6.5 Conclusions 171 - 172
6.6 Notation 172 - 173

7. OVERALL CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK
7.1 Overall conclusions 174 - 175
7.2 Suggestions for further work 175 - 176
7.2.1 Deposition of test micro-organisms on surfaces 175 - 176
7.2.2 Verification of the Zonal Shielding model 176
7.2.3 Deposition of test micro-organisms on surfaces 175 - 176
7.2.4 Verification of the Zonal Shielding model 176
7.2.5 Electrostatic spraying 177
7.2.6 Predicting impact rates from spray nozzles 177 - 178

**APPENDIX 1. COMPUTER PROGRAMS**

A1.1 Zonal Shielding model
   A1.1.1 Background 179 - 181
   A1.1.2 Program listing 181 - 193
A1.2 UV intensity programs
   A1.2.1 Introduction 193 - 194
   A1.2.2 Determination of UV lamp power output 194 - 196
   A1.2.3 UV intensities on objects of cylindrical geometry 196 - 202
   A1.2.4 UV disinfection tunnel 203 - 219

**APPENDIX 2. IMPACT TESTS IN THE SPRAY CHAMBER**

A2.1 Introduction 220
A2.2 Impact rate 220 - 223
A2.3 Electrostatic spraying 224 - 228

**APPENDIX 3. STATISTICS**

A3.1 Introduction 229
A3.2 Results 229 - 230

**REFERENCES** 231 - 240
LIST OF TABLES

1.1 Common sterilants and disinfectants

2.1 Stainless steel air-atomising nozzle characteristics

3.1 Multi-target kinetic constants for *B. subtilis* spore inactivation by UV
3.2 First-order kinetic constants for *B. subtilis* spore inactivation by UV
3.3 Comparison of multi-target parameters for *B. subtilis* spores in aqueous suspensions irradiated with UV
3.4 Summary of multi-target parameters for *Bacillus* spores exposed to 25.8% or 29% H₂O₂
3.5 Comparison of experimentally-determined disinfection levels to predicted disinfection levels from laboratory-based kinetics
3.6 Comparison of kinetic parameters for the UV and UV + 1.0% H₂O₂ process on different surfaces and in distilled water

4.1 Exposure factors for zonal shielding model
4.2 Results from the application of the zonal shielding model to beef steaks

5.1 Comparison of model results and bioassay results for the UV intensity in the spray chamber at different locations

A2.1 Effect of electrostatically-charging the spray on the impact rate on the outside of the stainless steel hollow cylindrical object
A2.2 Effect of electrostatically-charging the spray on the impact on surfaces of test objects
A2.3 Comparison of impact of spray on disc object using uncharged and electrostatically-charged sprays

A3.1 Multi-target kinetic constants for *B. subtilis* spore inactivation by UV
A3.2 First-order kinetic constants for *B. subtilis* spore inactivation by UV
A3.4 Summary of multi-target parameters for *Bacillus* spores exposed to 25.8% or 29% H$_2$O$_2$

A3.6 Comparison of kinetic parameters for the UV and UV + 1.0% H$_2$O$_2$ process on different surfaces and in distilled water
TABLE OF FIGURES

2.1 Microbial growth curves
2.2 *B. subtilis* spores on Grade 2 filter paper exposed to UV using the high intensity lamp
2.3 High intensity UV lamp arrangement
2.4 Low intensity UV lamp arrangement
2.5 Spray sterilisation chamber
2.6 End plates of the spray disinfection chamber
2.7 Method of attachment of test object to rotating shaft
2.8 UV lamp holder for spray sterilisation chamber
2.9 Test objects for use in the spray sterilisation chamber
2.10 Removable mounting for perspex spray nozzle
2.11 Electrostatic spray nozzle mounting lamp
2.12 *B. subtilis* spore-based UV intensity bioassay (on dry Grade 2 filter paper)
2.13 Production of aqueous ozone

3.1 Exposure of *B. subtilis* spores suspended in distilled water to UV
3.2 Exposure of *B. subtilis* spores on impervious surfaces to UV
3.3 Exposure of *B. subtilis* spores on Anodisc membranes to UV
3.4 Exposure of *B. subtilis* spores on dry Grade 2 and dry Grade 6 filter papers to UV
3.5 Exposure of *B. subtilis* spores on glass microfibre filter paper to UV
3.6 *B. subtilis* spores on Anodisc membranes
3.7 *B. subtilis* spores on Grade 2 filter paper
3.8 *B. subtilis* spores on Glass microfibre filter paper
3.9 Exposure of *B. subtilis* spores to UV on wet and dry Grade 2 filter paper
3.10 Exposure of *B. subtilis* spores to UV on wet and dry Grade 6 filter paper
3.11 Summary of disinfection curves of *B. subtilis* spores exposed to UV
3.12 Exposure of *B. subtilis* spores on PTFE coupons to 29 w/v% H₂O₂
3.13 Arrangement of *B. subtilis* spore impregnated Grade 2 filter paper strips on the disc and cylindrical objects for confirming experimentally-determined kinetics
3.14 *B. subtilis* spores on stainless steel coupons exposed to sprays of 29 w/v% H₂O₂

3.15 Disinfection performance for *B. subtilis* spores on Grade 2 filter paper exposed to UV and a range of concentrations of H₂O₂

3.16 Exposure of *B. subtilis* spores on Grade 2 filter paper to UV + 1.0 w/v% H₂O₂

3.17 Exposure of *B. subtilis* spores on Grade 6 filter paper to UV and UV + 1.0 w/v% H₂O₂

3.18 Exposure of *B. subtilis* spores on aluminium coupons to UV + 1.0 w/v% H₂O₂ in the spray chamber

3.19 Effect of spray pulse duration on disinfection performance for *B. subtilis* spores on aluminium coupons exposed to UV + 1.0 w/v% H₂O₂ in the spray chamber

3.20 *B. subtilis* spores on Grade 2 filter paper exposed to UV and aqueous O₃

3.21 *B. subtilis* spores on aluminium coupons exposed to gaseous O₃ (1.2%v/v)

3.22 Filter paper arrangement on hollow cylinder for electrostatic spraying experiments

3.23 *B. subtilis* spores on Grade 2 filter paper strips exposed to electrostatically-charged sprays of 29 w/v% H₂O₂

4.1 Example of the application of the zonal shielding model

4.2 Application of surface shielding model to the UV disinfection results obtained on dry Grade 2 filter paper strips

4.3 Application of surface shielding model to UV disinfection results obtained on dry Grade 6 filter paper strips

4.4 Application of surface shielding model to UV disinfection results obtained on Glass microfibre filter paper

4.5 Exposure factors for surfaces providing surface shielding

4.6 'Unshielded' disinfection data for beef flora (from the paper by Stermer et. al, 1987)

4.7 Disinfection data for beef steaks exposed to UV (from Stermer et. al, 1987)

5.1 Demonstration of view factors

5.2 Location of point sources in the UV source model
5.3 Arrangement for UV intensity measurements in the spray disinfection chamber using the radiometer
5.5 Object geometries in the spray disinfection chamber
5.6 UV intensity profiles on disc test object in the spray chamber produced by a single UV source
5.7 UV intensity profiles on disc test object in the spray chamber produced by 2 sources
5.8 UV intensity profiles on disc test object in the spray chamber produced by 3 UV sources
5.9 UV intensity profiles on disc test object in the spray chamber produced by 4 UV sources
5.10 UV intensity profiles on disc test object in the spray chamber produced by 2 UV lamps
5.11 UV intensity profiles on a cube in the spray chamber produced by 4 UV lamps facing the center of each side
5.12 UV intensity profiles on a cube in the spray chamber produced by 4 UV lamps facing the edges between the sides of the cube

6.1 Surface area zones of constant UV intensity
6.2 Disinfection tunnel arrangement
6.3 UV source arrangement on disinfection tunnel wall
6.4 Division of the disinfection tunnel into sections of constant UV intensity
6.5 UV intensity fields on surfaces of the slab in the disinfection tunnel
6.6 Disinfection level produced on slab-shaped object in disinfection chamber employing horizontal UV source arrangement
6.7 Disinfection levels produced on slab-shaped object exposed to UV in the disinfection chamber for different surface exposure factors
6.8 Disinfection level produced on slab-shaped object in the disinfection chamber when exposed to UV and 1.0 w/v% H2O2 spray of differing fractional surface coverage
6.9 Increasing the number of evenly-distributed UV sources on the walls of the disinfection tunnel
6.10 Disinfection level achieved in disinfection tunnel using different numbers of UV lamps per repeatable tunnel section
6.11 UV intensity on slab-shaped object in disinfection chamber using vertical UV source arrangement

6.12 Disinfection rate on slab using vertical UV source arrangement in disinfection tunnel

A2.1 Spray impact on the top of the PVC cylinder

A2.2 Spray impact rate on top of disc object under disinfection experimental conditions

A2.3 Spray impact on top of the disc object

A2.4 Impact on the side of the disc object from electrostatic and non-electrostatic sprays
1. INTRODUCTION

1.1 Surface disinfection

1.1.1 Definition of Terms

'Sterilisation' is commonly understood as implying the total destruction or elimination of all forms of life. 'Disinfection', on the other hand, has an altogether more restricted meaning i.e. that of freeing from infection. Block (1983) defines the term more specifically as 'destroying disease germs or other harmful micro-organisms or inactivating viruses'. He further goes on to state that disinfection agents are generally 'those that kill the growing forms, but not necessarily, the resistant spore forms of bacteria, except when the intended use is specifically against an organism forming spores'.

The context in which the term disinfection will be used throughout this work is simply in terms of processes or agents that result in either the total or partial elimination of certain organisms but not others. This definition maintains the distinction between sterilisation and disinfection without providing unnecessary constraints on the use of the latter term.

1.1.2 Scope of Application

Attention will be focused here on the disinfection of solid objects, as opposed to liquids or gases, which might harbour populations of harmful or otherwise unwanted micro-organisms. The particular concern of this work is those objects which human beings might come into contact with and which might act as potential sources of infection. Such objects in effect act as vehicles of infection. Excluded from consideration here is human skin; whilst frequently implicated as a vehicle of infection, particularly in hospital environments (Springthorpe and Sattar, 1990), its sensitivity requires specialised treatment which lies
outside the scope of this work.

In any consideration of the disinfection of a solid surface attention should be paid to the nature and properties of that surface. Contaminating micro-organisms will not necessarily be exposed at the top layers of contaminating surfaces. In porous materials for example, micro-organisms might be present deep in the body of the material, remote from the surface. Even surfaces which appear visibly smooth can contain microtopographic features which can provide protection to contaminating micro-organisms (Springthorpe and Sattar, 1990). Evidence exists to suggest that, in broad terms, the resistance of micro-organisms on a surface to disinfection treatments is often greater than the resistance of the same micro-organism when in suspension in liquid (Morris, 1972).

1.1.3 Disinfectants

There have been many techniques developed over several years for achieving sterilisation or disinfection of microbially-contaminated substances. These can be divided into two categories - physical agents and chemical agents. Table 1.1 lists the more common agents.

<table>
<thead>
<tr>
<th>Physical agents</th>
<th>Chemical agents</th>
</tr>
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<tbody>
<tr>
<td>Dry heat (hot air)</td>
<td>Salts</td>
</tr>
<tr>
<td>Moist heat (steam)</td>
<td>Acids and alkalis</td>
</tr>
<tr>
<td>Non-ionising radiation (UV)</td>
<td>Halogens and halogen-forming compounds</td>
</tr>
<tr>
<td>Ionising radiation</td>
<td>Oxidising agents</td>
</tr>
<tr>
<td>Filtration</td>
<td>Ether</td>
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<tr>
<td></td>
<td>Alcohols</td>
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<td></td>
<td>Soaps and detergents</td>
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<td></td>
<td>Phenols</td>
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<td></td>
<td>Formaldehyde</td>
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<tr>
<td></td>
<td>Glutaraldehyde</td>
</tr>
<tr>
<td></td>
<td>Ethylene Oxide</td>
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</table>

Table 1.1 Common sterilants and disinfectants (After Thomas (1988))
Naturally, no single agent is likely ever to be universally applicable in all situations. Treatments based on the application of moist heat at a temperature of 121°C, though generally effective would be wholly unsuitable for the treatment of a heat-labile material. Likewise, the use of certain chemical agents on foods would be inappropriate in instances where residual agent might itself prove harmful to health. The primary purpose of presenting Table 1.1 is to provide an indication of the wide range of treatments and agents which are available. Attention will focus in this work on the use of ultraviolet light and the oxidising agents, hydrogen peroxide and ozone used either by themselves or in combination with one another. Full descriptions on the use and efficacy of the other agents mentioned in the table may be found in Block (1983). Treatments which feature combinations of these agents are referred to as ‘advanced oxidation treatments’. These treatments will have particular advantages in certain applications but not in others. It is not the intention in what follows to identify every recorded application but generally to define broad areas where these techniques might hold future promise.

Food represents a particularly good example. Food can be a source of infection to humans due to contamination of the foodstuff by some external source or alternatively, the flora naturally associated with the surfaces of foods or within the foods can cause infection. Ingestion of microbially contaminated food can lead to food poisoning or food intoxication with consequences varying from gastric disturbances of varying degrees of severity to death. Much concern is being expressed in the microbiological contamination of fresh meat during slaughter and subsequent processing. This can be reduced by good sanitary practice but contamination of carcasses can still occur. Therefore there has been much interest in the disinfection of carcasses in abattoirs in order to maintain quality on storage, particularly in respect to beef (Reagan et al., 1973; Stermer et al., 1987; Greer and Jones, 1989; Kenney et al., 1995), although other studies have examined chicken (Banati et al., 1993), fish (Huang and Toledo, 1982) and pork (Fu et al., 1994).

Concern is not only directed towards meat; dried particulate foodstuffs, such as herbs and spices (Shama et al., 1994) and cereals and nuts (Andrews, 1996), can be a source of
infection due to micro-organisms present both within the particles and on their surfaces.

Food can also become contaminated by the packaging in which it is placed. A certain degree of contamination of the packaging might prove acceptable, as the levels on the product generally exceed those on the packaging by several orders of magnitude. However, the presence of even low numbers of certain pathogenic bacteria such as *Salmonella* are unlikely to be tolerated under any conditions. One area of food packaging that has received much attention is aseptic packaging, which is the process of placing a sterile product in a sterile container (Anon., 1980).

Considerable scope for UV and UV-associated disinfection treatments exists in hospitals and other health care environments. This ranges from instruments employed in general surgery and which may not, for whatever reason, be subjected to thermally treatment, such as endoscopes (Padial and Osborne, 1992), bone grafts and dialysis equipment (Thomas, 1988), equipment used in dental surgeries (Gurevich et al., 1996) to more advanced video-imaging medical equipment (Duppler, 1992). In addition, there may be a role for UV-based disinfection treatments in controlling the spread in hospitals of nosocomial infections such as methicillin resistant *Staphylococcus aureus* (MRSA) which have been shown to be dependent on a number of environmental factors (Ruef, 1995).

### 1.2 UV irradiation

#### 1.2.1 Introduction

The UV spectrum can be divided into 4 regions: UVA, from 400 to 315 nm, UVB, from 315 to 280 nm, UVC, from 280 to 200 nm, and Vacuum-UV, less than 200 nm (so termed because air strongly absorbs UV at wavelengths less than 200 nm, therefore making its use only practical under vacuum conditions; Meulemans, 1987).
The use of ultraviolet light for the destruction of micro-organisms was observed in the early 20th century but was never adopted at that time as a practical method of sterilisation due to the low power of the UV lamps available. In the 1970's UV sterilisation became of interest again as new legislation controlling chemical residues in food packaging came into force and new higher power UV lamps became available (Bachman, 1975; Sugawara et al., 1981).

The germicidal effectiveness of different UV wavelengths for a particular organism may be directly compared in a so-called ‘inactivation action spectrum’. Peak germicidal activity is generally found to be at a wavelength of 260 nm, which is close to the peak line of a mercury vapour lamp (Munakata et al., 1991).

1.2.2 Disinfection with UV

Ultraviolet light (UV) is an effective disinfectant for a wide range of micro-organisms and is used for the disinfection of water (Shama, 1992) and the disinfection of air (Nakamura, 1987). There are many other potential applications for UV as a disinfectant. UV has been proposed as a disinfectant for food packaging (Bachman, 1975; Toledo et al., 1973). It has also been proposed as a disinfectant for foodstuffs, including meat and fish.

Huang and Toledo (1982) investigated the use of UV as a disinfectant for the surface of whole fish. They found UV to be an effective disinfectant when applied to smooth-surfaced fish such as Spanish mackerel. However, UV was not very effective when used on rough-surfaced fish, such as mullet and croaker. The authors attributed this to shadow effects caused by the rough surface which shielded the natural flora from the incident UV.

In a study on the use of UV for the disinfection of beef carcasses, Stermer et al. (1987) found that UV reduced the concentration of natural flora on beef steaks. The natural flora, when cultured and grown on agar plates, was disinfected more quickly on agar plates than on the beef steaks, suggesting that the surface afforded some protection to the flora.
Reagan et al. (1973) showed that UV irradiation produced an increased lag phase in the microbial growth curve on beef tissue and also produced a reduced growth rate after the initial lag phase. Greater disinfection levels were achieved on fatty tissue than on lean muscle tissue. Kaess and Wiederman (1973) showed that UV was an effective disinfectant for chilled beef slices when the UV intensity was greater than 2 μW/cm². These workers found that the application of UV extended the initial lag phase in the growth of *Pseudomonas*, and the moulds *Thamnidium* and *Penicillium*. The microbial growth rate of *Pseudomonas* was also reduced but the growth rate of the moulds was unaffected.

Hirose et al. (1982) investigated the effects of high intensity UV (of between 2 - 60 mW/cm²) on the disinfection of the surface of Wiener sausages. They found that whilst UV was an effective disinfectant against *E.coli* and *B.subtilis* spores in suspension, the disinfection rate for *E.coli* on the surface of the sausage obtained was only 0.019 times that obtained in suspension. In addition, it was observed that less disinfection was obtained at the ends of the sausage, which were creased. This was attributed to shadow effects.

Lee et al. (1989) showed that UV could be used to disinfect *Salmonella* in molten chocolate if the chocolate was less than 1 mm thick. It was found that the milk chocolate attenuated the UV, causing the disinfection level to be reduced as the thickness of the chocolate film increased even whilst keeping the UV intensity constant.

Shama et al. (1994) used UV to inactivate *B.subtilis* spores on ballotini which were pneumatically conveyed through a UV field. Such a method could be applied to the disinfection of herbs and spices, as well as various pharmaceuticals and cosmetics.

Van der Molen et al. (1980) showed that UV could be used to reduce the numbers of viable phototrophic bacteria growing in a biofilm on a stone church wall when applied continuously for one week. It was found that the UV had to be applied in one dose to be effective. If the UV was applied only during the night then the bacteria were able to repair themselves during the daytime and little reduction in viable bacterial numbers was achieved.
1.2.3 Disinfection Kinetics

Inactivation curves are typified by characteristic phases; the first of these is the initial lag phase. The disinfection rate then gradually increases and 1st order kinetics are generally obtained (order in this case being with respect to microbial concentration). The portion of the curve prior to the 1st order section is commonly referred to as the 'shoulder' of the curve. Disinfection curves are often referred to as being 'shouldered 1st order kinetic curves'. The shoulder of the disinfection curve is attributed to the presence of mechanisms natural to the micro-organisms which can repair a limited amount of cellular damage up to a certain level and beyond which inactivation ensues. At higher levels of disinfection (over 3 orders of reduction in the viability) the inactivation rate gradually reduces, sometimes giving rise to a stationary phase at between 4 and 5 orders of reduction in viability. This has been attributed to a small fraction of UV resistant cells (Sugawara et al., 1981).

UV inactivation models assume light to be comprised of photons. Photons of UV light are considered to strike the medium in which the micro-organisms are present. Some of these photons hit the micro-organisms and are absorbed by them. Only a fraction of the photons absorbed will actually cause death or inactivation of cells. This fraction is known as the 'quantum yield' (Harm, 1980a). The 'observed quantum yield' is reduced by the repair mechanisms in the cells. Therefore the observed quantum yield is always lower than the underlying quantum yield.

In any irradiation event, the level of inactivation of micro-organisms is related to the quantity of UV that the micro-organisms are exposed to. The term 'fluence', has been adopted and is the product of UV intensity and time (Harm, 1980b).

Two types of UV inactivation kinetics have been proposed: 'multi-target' and 'multi-hit' kinetics. Multi-target kinetics assume that there are a certain number of targets in a cell that must each be struck by a photon to inactivate the cell (Harm, 1980c). Multi-target type inactivation curves are generally applied to cases where suspended cells exist naturally in small clusters or where cells contain multiple sets of their genetic material. Multi-hit kinetics
assume that a certain target must be hit a certain number of times for the cell to be inactivated (Harm, 1980c).

The following expression has been developed for multi-target kinetics (Harm, 1980c):

\[
\left( \frac{N}{N_0} \right) = 1 - (1 - e^{-kF})^n
\]

where

- \( N \) = microbial count
- \( N_0 \) = initial microbial count
- \( K \) = inactivation rate coefficient
- \( F \) = UV fluence = Intensity x Exposure time
- \( n \) = number of targets

This expression can be simplified at low survival levels by applying the binomial theorem to obtain:

\[
\left( \frac{N}{N_0} \right) \approx ne^{-kF}
\]

This expression can be plotted on a semilog graph of surviving fraction against fluence. This gives the log of the number of targets, \( \log n \), as the intercept and the inactivation rate coefficient, \( k \), as the gradient.

In practice not all inactivation curves follow the multi-target type. In some cases the target numbers obtained are not integers. This occurs as a result of the cells’ repair mechanisms and no truly general equation for UV inactivation kinetics can be produced as the repair mechanisms active in any given situation will depend on many factors such as the species involved, the nature of the environment etc. UV inactivation kinetics are usually classified either by a decimal reduction time (the time taken to inactivate 90% of cells based on first order disinfection kinetics). This value is supplemented by an 'extrapolation number'. The extrapolation number is the intercept on the \( \log_{10}(N/N_0) \) axis of a semilog plot of...
microbial survival against fluence when extrapolating the first-order part of the disinfection curve. This is equivalent to the target number if multi-target kinetics were observed.

It should be noted that these models do not account for the tailing of the inactivation curves which is sometimes observed. Sugawara et al. (1981) adapted a multi-hit model for the UV inactivation of *B.subtilis* spores by postulating that there existed a small fraction of UV resistant spores which had the same number of hits required for inactivation as the remainder of the population. This model was fitted successfully to their experimental data.

Caution must be exercised in using these data. For example, it has been shown that the growth medium can greatly affect the UV-resistance of *B.subtilis* spores. This could be due to proteins within the spore protoplast, either through direct absorption of UV or by binding to the DNA, thus affecting its UV absorption. The quantity of proteins in a spore will be affected by the growth medium (Bayliss et al., 1981). Due to the variation in the disinfection performance of UV against the same micro-organisms in studies by different researchers such tables can only be used as a guide to the approximate UV dose required. An example of such a table is given by Chang et al. (1985).

1.2.4 Mechanism of UV-induced inactivation

The germicidal action of ultraviolet light has been shown to be due to absorption by the nucleotide bases in microbial DNA, mainly by the pyrimidine bases, causing the formation of harmful ‘photoproducts’. These photoproducts alter the DNA of the micro-organism and prevent replication. The main photoproducts of biological effect are all pyrimidine derivatives - cyclobutyl-type dimers, pyrimidine adducts, pyrimidine hydrates, and DNA-protein crosslinks. One specific product, 5-thyminyl 5-6-dihydrothymine - often referred to as the spore photoproduct, is formed in spores and is the dominant cause of their inactivation (Harm, 1980d). For high intensity UV (10^{11} - 10^{13} Wm^{-2}) it has been shown that the dominant cause of inactivation in viruses and bacterial plasmids is single-strand DNA breaks (Gurzadyan et al., 1981).
Most studies into the germicidal effects of UV have been concentrated at wavelengths below 300 nm. However it has been found that 365 nm UV (near UV) can have an inactivating effect on micro-organisms (Sammartano and Tuveson, 1985; Eisenstark et al., 1986; Yallaly and Eisenstark, 1990). The mechanism involved must be different to that involved with far UV as nucleic acids only weakly absorb energy from near UV. It has been claimed that near UV causes the formation of hydrogen peroxide in situ in the micro-organisms (Yallaly and Eisenstark, 1990).

1.2.5 Repair and recovery of UV-induced damage

1.2.5.1 Repair mechanisms for UV-induced damage

Virtually all micro-organisms are capable of recovering from UV-induced injury due to the possession of mechanisms capable of repairing damage within the cells (Harm, 1980e). The cell component most vulnerable to damage is the DNA. This is because there is only one copy per cell and it is a large molecule. All known molecular repair processes act upon nucleic acids, in particular DNA. This is thought to be due to the fact that other important molecules in cells exist in many copies and therefore damage to a small fraction of these will not seriously affect cellular functions. Ultimately, repair of all cell components is governed by the cells genome.

The repair mechanisms for UV-induced damage of cell DNA can be classified according to the method by which the DNA is repaired. These are: reversal of the UV-induced alteration, replacement of UV-damaged nucleotides, and combination of undamaged regions in replicating DNA molecules.
1.2.5.2 Photoreactivation and Photoenzymatic repair

This class of repair mechanism reverses the UV-induced alteration. The damage caused to cells exposed to far UV irradiation can be reduced when the cells are exposed to light in the near UV or violet-blue range of the spectrum (310 - 480 nm), and is sometimes referred to as 'photoreactivating light' and the process of repair 'photoreactivation'. Exposure to far UV can result in the formation of cyclobutyl pyrimidine dimers. An enzyme, referred to as a 'photolyase', becomes activated when exposed to light of the appropriate wavelength and acts to hydrolyse the dimers. Other repair processes that do not use light are often referred to as 'dark repair' mechanisms, such as those mechanisms discussed in sections 1.2.5.3 and 1.2.5.4.

1.2.5.3 Excision - Resynthesis Repair

This class of repair mechanism involves the removal of the damaged sections of the DNA (excision), the resynthesis of the removed sections and rejoining those sections into the DNA. An example of this repair is in the removal of pyrimidine dimers from DNA damaged by far UV. The damaged regions of the cell DNA are recognised by a 'repair endonuclease' which cleaves the polynucleotide strand by the dimer. The cleaved sections of the DNA are soluble in acid and hence gradually dissolve, leaving single-stranded sections in the DNA. The complementary strand in the single-stranded DNA sections acts as the template for the resynthesis of the missing sections of the DNA. The resynthesised DNA sections are rejoined to the DNA by the enzyme polynucleotide ligase (Harm, 1980f).

These mechanisms are all enzyme-based and in total there are at least four enzymatic activities that have to be performed. The precise mechanisms comprising each of these stages of the excision-resynthesis repair process vary between organisms (Harm, 1980f).
1.2.5.4 Recombination repair

This class of repair mechanism combines separate parts of DNA strands to form one complete DNA molecule. For any damaged section of the DNA, if the adjacent section on the complementary strand is undamaged then the genetic code remains intact to resynthesise the damaged section of the DNA. Before recombination repair can take place it is necessary for the cell to replicate the DNA. When the cell attempts to replicate the DNA it is not possible to replicate the damaged sections of the DNA. This leads to the formation of separate sections of the DNA molecule, in addition to the strands of the original DNA molecule. The damaged sections along the DNA strands are then replaced by the newly created DNA sections and the two single strands of the DNA recombine.

As the recombination repair mechanism can only occur after replication of the damaged DNA it is sometimes referred to as 'postreplication repair' (Harm, 1980g).

1.2.5.5 Repair mechanisms for damage caused by other sterilants

The classes of DNA repair mechanisms described for UV-induced damage also apply to other sterilants which damage cell DNA, e.g. hydrogen peroxide or hydrogen peroxide combined with UV. Hydrogen peroxide and ozone are also known to act on other cell constituents, such as the cell wall or membrane (Block, 1983; Stevenson and Shafer, 1983). Naturally, repair to cellular constituents other than nucleic acids requires the cells’ DNA to be intact.
1.3 Peroxidation

1.3.1 Introduction

Hydrogen peroxide ($\text{H}_2\text{O}_2$) has been used as a bactericidal and sporicidal agent for several decades. At low concentrations it is bactericidal but not sporicidal. The majority of studies have employed a hydrogen peroxide concentration greater than 10 w/v\% to achieve a sporicidal effect, with one notable exception being Baldry (1983) who reported sporicidal action using 2.6 w/v\% $\text{H}_2\text{O}_2$ for contact times of over four hours. In general, the sporicidal efficiency increases with hydrogen peroxide concentration (Stevenson and Shafer, 1983).

Hydrogen peroxide solutions have been recommended for the disinfection of surgical implant components, temperature-sensitive plastic equipment, hydrophilic soft contact lenses, commercial packing materials, water, and milk (Klapes and Vesley, 1990).

$\text{H}_2\text{O}_2$, like other chemical sterilants, is constrained in its use by legislation. For example, the 'FDA' set a maximum allowable $\text{H}_2\text{O}_2$ concentration in foods to be 0.1 ppm. Thus if $\text{H}_2\text{O}_2$ is used as a sterilant for food packaging, the residual $\text{H}_2\text{O}_2$ must be controlled. $\text{H}_2\text{O}_2$ is quite stable at ambient temperatures. Therefore processes using $\text{H}_2\text{O}_2$ as a surface sterilant often operate at temperatures above ambient and use hot air to remove residual hydrogen peroxide (Stevenson and Shafer, 1983).

Hydrogen peroxide vapour has also been proposed as a surface sterilant. Two methods for its production are given - sparging air through aqueous hydrogen peroxide (Wang and Toledo, 1986) or by injecting aqueous hydrogen peroxide into a low pressure chamber (Klapes and Vesley, 1990). The use of vapour-phase hydrogen peroxide has the advantage over liquid hydrogen peroxide that it will be applied more uniformly. Also, it avoids the problems of forming films and pools of hydrogen peroxide on the treated surface which can cause hydrogen peroxide to be adsorbed to the surface or to sub-surface material. Hydrogen peroxide vapour has the additional advantage that it is non-toxic in contrast to
previously used gaseous sterilants such as ethylene oxide and formaldehyde (Klapes and Vesley, 1990).

1.3.2 Disinfection using hydrogen peroxide

There has been a great deal of interest in the inactivation kinetics of micro-organisms using H₂O₂ as the sterilant e.g. (Toledo et al., 1973; Bayliss and Waites, 1981; Stevenson and Shafer, 1983; Leaper, 1984). These papers proposed H₂O₂ as a surface sterilant for aseptic packaging. The test micro-organism used in these studies was typically B.subtilis spores (various strains) as this organism has been shown to be one of the most resistant to hydrogen peroxide (Toledo et al., 1973). In these studies, spores were either used directly by suspending them in hydrogen peroxide solutions or else were placed on filter paper strips prior to immersion in hydrogen peroxide solutions.

The curves of H₂O₂-induced microbial inactivation are similar in shape to those of UV inactivation. H₂O₂-induced microbial inactivation has usually been described by decimal reduction times even though the disinfection kinetics (with respect to microbial concentration) are not first order. They are shouldered first order, sometimes with a stationary phase after 4-5 orders of inactivation (Toledo et al., 1973; Cerf and Metro, 1977; Bayliss and Waites, 1981; Bayliss et al., 1981; Stevenson and Shafer, 1983; Wang and Toledo, 1986). Cerf and Metro (1977) investigated the tailing of the inactivation curves of B.licheniformis spores treated with hydrogen peroxide. They were able to eliminate the possibility of a mutant H₂O₂-resistant strain being formed during cultivation by growing colonies from a single spore. They concluded that the tailing effect was due to clumping of the spores. The affinity which the spores had for each other led to the formation of clumps of more than one hundred spores, with diameters greater than six spore widths. Catalase naturally present in the spores decomposes H₂O₂; it was found that the catalase was inactivated but only at a very slow rate. Therefore, viable spores are afforded protection from hydrogen peroxide when they are surrounded by inactivated spores because the latter act as a sink for hydrogen peroxide due to the catalase still present in them.
The disinfection performance of hydrogen peroxide is greatly increased with temperature (Toledo et al., 1973). The effect of a 10°C rise in temperature has been shown to cause the disinfection rate due to hydrogen peroxide to increase to between 1.6 and 2.5 times its original value. The temperature dependence of the microbial disinfection rate due to hydrogen peroxide can be described by the Arhenius equation.

The relationship between hydrogen peroxide concentration and the inactivation rate has repeatedly been shown not to be linear (Toledo et al., 1973; Stevenson and Shafer, 1983; Leaper, 1984; Wang and Toledo, 1986). In the absence of a mechanistically-based model, Leaper (1984) fitted an empirical model to the relationship.

### 1.3.3 Mechanisms of microbial inactivation

Working with DNA extracted from *Bacillus subtilis* (60009), Rhaese and co-workers (Rhaese and Freese, 1968; Rhaese et al., 1968) found that hydrogen peroxide caused the breakage of double bonds in DNA causing the liberation of all four bases (some of which were altered), and broke the sugar-phosphate backbone. Massie et al. (1972) also found base destruction by H\(_2\)O\(_2\) in calf thymus DNA and in addition detected single-strand and double-strand breaks and cross-linking in the DNA. They found that base destruction was the dominant mechanism, proceeding at more than twenty times the rate of formation of single-strand breaks. Indeed, base destruction by H\(_2\)O\(_2\) was so high that absorbance of 260 nm UV was reduced. H\(_2\)O\(_2\) reacts more readily with adenine and thymine nucleotides than it does with guanine and cytosine nucleotides.

In addition to its effect on DNA, it has also been shown that hydrogen peroxide destroys proteins in spores (Stevenson and Shafer, 1983) with the primary target being the spore coat. Bayliss and Waites (1976) showed that treatment with sub-lethal doses of hydrogen peroxide actually stimulated germination.
The effect of metal ions, particularly cupric ions, on the disinfection performance of hydrogen peroxide has been widely reported. Massie et al. (1972) found that the addition of CuCl₂ increased the rate of base destruction by H₂O₂ whilst FeCl₂ had little effect and MgCl₂ decreased the rates. Salts which are effective in increasing the oxidising ability of hydrogen peroxide will therefore increase the bactericidal effect of hydrogen peroxide. The most effective catalyst for hydrogen peroxide disinfection is Cu²⁺ ions, with Fe²⁺ ions also noted for a catalysing effect by some researchers (Stevenson and Shafer, 1983). Stark and Faber (1985) found that iron and copper ions were required for hydrogen peroxide to be lethal to cultured hepatocytes, whereas Bayliss and Waites (1981) found that the resistance of Serratia marascens to H₂O₂ to be unaffected by the cellular iron content but demonstrated the additional lethality of H₂O₂ to C.bifermentas spores in the presence of Cu²⁺ ions (Bayliss and Waites, 1976). Therefore the results of Bayliss and Waites agree with the findings of Massie et al. (1972) that Cu²⁺ ions are a more important factor in H₂O₂ lethality than Fe²⁺ ions. The combination of hydrogen peroxide and Fe²⁺ ions is called 'Fenton's reagent'.

Several factors are known to influence the resistance of micro-organisms to hydrogen peroxide. One of these is the growth medium. Bayliss et al (1981) grew B.subtilis cultures using different growth media. They showed that the growth media had a marked effect on the surviving fraction of micro-organisms after treatment with hydrogen peroxide. However, no relationship between spore coat properties and hydrogen peroxide resistance could be found. The growth media was also shown to affect the number of cells surviving treatment with UV. However the effect of growth medium on survival was different for the two types of disinfection treatment.
1.4 Ozonation

1.4.1 Introduction

Ozone is a highly reactive oxidant and disinfectant in both gaseous and aqueous form. Aqueous ozone has been widely investigated for water treatment, both as a disinfectant and as an oxidant for the destruction of various pollutants. An intensive literature search only uncovered one previous study (that of Rickloff (1987)) in which aqueous ozone had been used specifically as a surface sterilant. One of the difficulties of working with aqueous ozone is that it is subject to losses through decomposition and reactions with various impurities in water. This has led to wide disagreement about disinfection rates and kinetics between previous researchers due to errors in controlling and measuring ozone concentration (Zhou and Smith, 1994).

1.4.2 Disinfection using ozone

Ozone inactivation has been described in terms of first order kinetics. Some researchers have observed an initial lag phase in the disinfection curves (Muraca et al., 1987; Wickramanayake and Sproul, 1988). Tailing of the curves has also been noted (Zhou and Smith, 1994). The tailing of the inactivation curves from ozone is attributed to the formation of clumps of micro-organisms, as was the case for disinfection by hydrogen peroxide. This theory was given support by the work of Dahi (1976) who showed that after breaking up the clumps of micro-organisms by ultrasound, first order inactivation kinetics were obtained. The rate of disinfection using aqueous ozone is rapid and this makes it difficult to obtain kinetic data from the initial part of the curve. This might explain why some researchers have not reported the existence of an initial lag phase (Zhou and Smith, 1994). In general terms, inactivation curves obtained using $O_3$ show similar characteristics to those for UV and $H_2O_2$-induced microbial inactivation.
Whilst it has been shown that the inactivation kinetics are nearly first order with respect to microbial concentration, the order with respect to ozone is almost certainly much greater than one according to Zhou and Smith (1994). A figure in the region of 3 has been suggested (Glaze et al., 1987). It has been shown that the microbiocidal activity of ozone occurs at the surface of micro-organisms, leading to the disintegration of the cell wall (Zhou and Smith, 1994). Therefore the order of the inactivation kinetics will be governed by the interactions of ozone molecules and the microbial envelope. With other disinfectants where the microbiocidal activity is due to effects within the cytoplasm, the disinfection kinetics are often mass transfer limited and hence the order of the disinfection kinetics with respect to the disinfectant concentration are close to unity (Zhou and Smith, 1994). As ozone primarily acts at the surface, the disinfection rate will be determined by the reaction rate of ozone with the cell wall and this explains why the disinfection kinetics are so strongly dependent on ozone concentration. In contrast to this, Wickramanayake and Sproul (1988) found the order of the disinfection kinetics of *N. gruberi* cysts with respect to ozone concentration to be near unity. This would suggest that the biocidal effect of the ozone does not occur at the surface of the cysts.

Rickloff (1987) investigated the use of aqueous ozone for sterilising solid surfaces by immersing porcelain cylinders, onto which bacterial spores had been dried, into aqueous ozone. It was found that aqueous ozone of approximately 10 mg/L at 20°C was effective against spores in suspension and against spores dried onto a clean surface. However aqueous ozone proved ineffective for inactivating spores surrounded by organic material on a solid surface, such as soil. Raising the temperature to 60°C made the disinfection performance worse in the presence of soil.

The disinfection rate of micro-organisms by aqueous ozone has been shown to be influenced only slightly by temperature (Muraca et al., 1987; Zhou and Smith, 1994). Beltran et al. (1994) showed that increasing the temperature of aqueous ozone produced two conflicting effects. Increasing the temperature increased the reaction rate constant but this was countered by a decrease in the aqueous ozone concentration as the saturated concentration decreased with temperature. When the aqueous ozone concentration was
kept constant, an increase in the oxidation rate due to aqueous ozone was found. The inactivation rates' dependence on temperature has been adequately described using the Arhenius equation (Wickramanayake and Sproul, 1988; Zhou and Smith, 1994).

Ishizaki et al (1986) investigated the effect of ozone gas on six species of Bacillus spores. The inactivation kinetics observed were shouldered first order (except for B.cereus which displayed no shoulder). Using ozone concentrations of between 0.5 and 3.0 mg/l it was found that the relative humidity of the ozone gas used greatly influenced the inactivation rate. Relative humidities in excess of 50% were needed for significant inactivation at these ozone concentrations. Increasing the relative humidity increased the first order kill rate and decreased the lag time. These findings would seem to suggest ozone is only sporicidal in the presence of water.

1.4.3 Mechanism of disinfection

Whilst, as the foregoing suggests, the use of ozone as a disinfectant is now becoming widely established, the cellular targets against which ozone or its breakdown products act, have not been unambiguously identified. These cellular targets are in any event likely to be non-specific and to be dependent on cell type, the physiological state of the cell, etc. This is in sharp contrast to UV irradiation where the primary lethal effect is directed against the cells' genome. What is incontrovertible is that ozone exerts its lethality through the generation of free radicals. The processes by which this occurs has been extensively studied in relation to the oxidation of (relatively simple) chemical structures. Therefore it is appropriate to give some considerations to previous studies in which the mechanism of free radical generation has been investigated because such processes will be relevant to cellular inactivation.

Ozone and its breakdown products can react directly with chemical species present in water (Staehelin and Hoigne, 1985). The decomposition of aqueous ozone is due to a chain reaction involving $OH^-$ radicals. This chain mechanism is affected by the presence of
species which scavenge the $OH^-$ radicals and hence inhibit the chain mechanism, and also by species which promote the chain by converting $OH^-$ radicals (which react with many chemical species) to $O_3^-$ radicals. The latter mainly react with $O_3$, hence promoting further reaction. In very pure water, hydroxyl radicals react with ozone so that the chain propagating steps shown can be repeated several times. It has been postulated that many hundreds of ozone molecules may be decomposed by a single initiation step (Glaze et al., 1987).

The presence of these promoters and inhibitors greatly influences the rate of ozone decomposition and, as a consequence, the concentration of the ozone and its decomposition products, which in turn affects the reaction rates of the processes of interest. The nature of specific promoters and inhibitors and their concentrations will vary between different water sources. Therefore results conducted by different groups do not generally agree well, even with respect to the kinetic orders of the reactions. Typical promoters are formic acid, methanol and phosphate ions. Typical $OH^-$ radical scavengers include alkyl groups, t-Butyl alcohol, and bicarbonate/carbonate ions (Staehelín and Hoigne, 1985). By treating the effects of promoters and inhibitors in general, these workers show that the rate of ozone decomposition is first order with respect to ozone, independent of the number of promoters and inhibitors. This was not the case where the radical formation becomes fast, e.g. under high alkaline conditions or in the presence of UV. The rate of ozone decomposition with respect to ozone concentration can be between half to second order under such conditions (Staehelín and Hoigne, 1985). The relative rates of these reactions, in conjunction with the decomposition rate of ozone, will affect the inactivation kinetics obtained. Thus, unless identical conditions are employed every time, it will be difficult to make an accurate prediction of ozone-induced microbial inactivation kinetics.

The mechanism for the decomposition of aqueous ozone proposed by Staehelín and Hoigne (1985) is shown below:

20
Initiation step:

\[ O_3 + OH^- \rightarrow HO_2^* + O_2^- \]  

(1)

Propagation steps:

\[ HO_2^* \Leftrightarrow O_2^- + H^+ \]  

(2)

\[ O_2^- + O_3 \rightarrow O_3^- + O_2 \]  

(3)

\[ O_3^- + H^+ \rightarrow HO_3^* \]  

(4)

\[ HO_3^* \rightarrow OH^* + O_2 \]  

(5)

\[ OH^* + O_3 \rightarrow HO_2^* + O_2 \]  

(6)

Termination steps:

\[ 2OH^* \rightarrow H_2O_2 \]  

(7)

\[ OH^* + M \rightarrow \text{products} \]  

(8)

where M is a radical scavenger

Aqueous ozone decomposes to several oxidising species e.g. hydrogen peroxide, hydroxyl radicals and superoxide radicals. Much of the work conducted into disinfection has not concerned itself with the particular chemical species responsible for the oxidation of the biological material. However such studies have been undertaken for the oxidation of various pollutants in water. Although these studies are not concerned with disinfection, they provide useful information about the oxidation mechanisms of ozone, which is relevant because the disinfection properties of ozone are attributed to its oxidising micro-organisms, rendering them inactive.

Beltran et al. (1994) investigated the oxidation of azatrine by ozone. By employing the radical scavenger, t-butanol, it was found that oxidation was primarily due to radicals, even at pH 2 where the radical concentration is much lower than at pH 7. Consequently increasing the pH increases the oxidation rate for a given aqueous ozone concentration.
Nonetheless, these workers showed that ozone reacted directly with azatrine but the reaction rate was much lower than that for the radicals.

When employing aqueous ozone as a sterilant it is necessary to know the decomposition rate of the aqueous ozone so that a suitable aqueous ozone concentration and contact time can be maintained. The decomposition rate of aqueous ozone can be accurately predicted under acidic conditions using the equation developed by Morooka et al. (1978).

1.5 Combined treatments (Advanced oxidation methods)

1.5.1 Introduction

Interest has been shown in the use of combinations of UV, H₂O₂ and O₃ as sterilants. These combinations have been shown to have a greater oxidising effect than the sum of the oxidising effects of the individual oxidants, i.e. a synergistic effect is produced. From studies of these processes, the synergism has been attributed to the formation of intermediate hydroxyl radicals which are highly reactive and hence short-lived. The hydroxyl radical is the most powerful oxidising species after fluorine. Its oxidising action is mostly brought about by hydrogen abstraction (Legrini et al., 1993). Due to the very fast reaction rate coefficients for reactions involving hydroxyl free radicals, the reaction rate is often controlled by the rate of formation of hydroxyl radicals (Wolfrum et al., 1994).

Processes that form hydroxyl radicals as a result of a combination of treatments are referred to as 'Advanced Oxidation Processes'. This class of process covers all the permutations of UV, H₂O₂ and O₃ and also includes other processes in which hydroxyl radicals are formed. The latter include aqueous ozone at high pH values and ozone or hydrogen peroxide with other hydroxyl radical initiators such as metals and metal oxides (Glaze et al., 1987).

The hydroxyl free radical is difficult to detect directly because it is so short-lived. It has
been detected by electron paramagnetic resonance (EPR) spectroscopy when employing a 'trap' for the hydroxyl radicals (Wolfrum et al., 1994). It should be noted though that direct evidence for the existence of the hydroxyl free radical has not been obtained but there is a body of indirect evidence for its existence.

1.5.2 UV and hydrogen peroxide

1.5.2.1 Disinfection

Bayliss and Waites (1979) investigated the simultaneous use of hydrogen peroxide and far UV. They found that the simultaneous use of hydrogen peroxide and UV produced a synergistic inactivation effect against *Bacillus subtilis* spores. Interestingly, no synergistic effect was obtained when hydrogen peroxide and UV were used successively. Bayliss and Waites (1981) noted that when hydrogen peroxide was used after UV, the resulting level of inactivation was less than the sum of the 2 individual treatments. They explained this effect by the breaking of spore clumps and possible reversal of DNA damage by H$_2$O$_2$.

Bayliss and Waites (1979) found an optimum inactivation rate was obtained with a H$_2$O$_2$ concentration of 0.3 M (= 1.0 w/v%) for the combined treatment for *B. subtilis* (NCDO 2129) spores. Although the UV intensity employed was not stated, it can be taken to have been about 260 μW/cm$^2$ as the physical arrangement appears identical to previous work of theirs (Bayliss and Waites, 1980). In this work, the combined treatment was used against vegetative cells. Results from H$_2$O$_2$ concentrations above 1.0 w/v% were not presented but the authors claimed that 1.0 w/v% was the optimum concentration for the UV + H$_2$O$_2$ process for vegetative cells. The UV intensity will affect the quantum yield of H$_2$O$_2$ decomposition (Lunak and Sedlak, 1992) and so 1.0 w/v% H$_2$O$_2$ may not represent a universal optimum for all UV intensities. However Bayliss and Waites (1982) did investigate the UV + H$_2$O$_2$ process with a much higher intensity UV source giving an intensity of 1.8 mW/cm$^2$ compared to the 260 μW/cm$^2$ used previously. For spores from the same strain of *B. subtilis*, the optimum H$_2$O$_2$ concentration was found still to be about
1.0 w/v%. For a different strain of *B. subtilis* spores (NCDO 2130) a weaker optimum existed in the range 1.0 - 2.5 w/v%. On the basis of this result, similar H$_2$O$_2$ concentrations were henceforth used in their studies. Bayliss and Waites attributed this optimum to the fact that at higher hydrogen peroxide concentrations more UV would be absorbed by the hydrogen peroxide, thus reducing the UV intensity near the spores. This is supported by their finding that doubling the volume of liquid reduced the kill. For hydrogen peroxide concentrations of over 5 w/v%, Bayliss and Waites showed that combining UV with H$_2$O$_2$ gave a lower disinfection performance than UV alone.

Stannard *et al.* (1983) showed that synergism was observed when combining UV with hydrogen peroxide when used against four micro-organisms which had been applied to the surfaces of food cartons. Hydrogen peroxide was sprayed onto the inside of the cartons which were subsequently exposed to 10 seconds of UV (dose not given). The maximum level of synergism was achieved with hydrogen peroxide concentrations in the range of 0.5 to 1.0 w/v%.

**1.5.2.2 Mechanisms of microbial inactivation and hydroxyl radical formation**

The synergistic inactivation effect has been attributed to the formation of hydroxyl radicals. It is known that hydroxyl radicals attack DNA in cells (Adinarayana *et al.*, 1988; Dirksen *et al.*, 1988). A large number of products from both the sugar moiety and the heterocyclic bases in DNA have been isolated and identified. Hydroxyl radicals induce both DNA strand breaks and base release (Glaze *et al.*, 1987).

The inactivation kinetics obtained using UV and hydrogen peroxide are due to at least three separate sterilants - UV and hydrogen peroxide and the decomposition products of hydrogen peroxide - notably hydroxyl free radicals. To understand the mechanism of inactivation using UV and hydrogen peroxide it is necessary to understand the decomposition of hydrogen peroxide in the presence of UV (sometimes referred to as 'photodecomposition').
It has been known for several decades that hydrogen peroxide decomposes in the presence of ultraviolet light to water and oxygen. Urey et al. (1929) suggested that this was due to the formation of hydroxyl radicals.

\[ \text{hv} \]
\[ H_2O_2 \rightarrow 2OH^* \]  

Haber and Weiss suggested that the hydroxyl radicals formed should bring about a radical chain mechanism in which the propagation cycle gives high quantum yields (Lunak and Sedlak, 1992):

\[ \text{OH}^* + H_2O_2 \rightarrow H_2O + HO_2^* \]  
\[ HO_2^* + H_2O_2 \rightarrow O_2 + H_2O + OH^* \]  

However high quantum yields are not always obtained due to the following termination steps:

\[ \text{HO}_2^* + \text{OH}^* \rightarrow \text{O}_2 + H_2O \]  
\[ 2\text{HO}_2^* \rightarrow H_2O_2 + O_2 \]  
\[ 2\text{OH}^* \rightarrow H_2O + O \]  
\[ 2\text{OH}^* \rightarrow H_2O_2 \]  
\[ \text{OH}^* + X \rightarrow \text{interruption of the chain} \]

where \( X \) is a radical scavenger.
A linear dependence of quantum yield both on the concentration of hydrogen peroxide and on the reciprocal value of the square root of the intensity of absorbed radiation has been found but this relationship has only been tested over narrow ranges (Lunak and Sedlak, 1992).

The quantum yield of the photolysis of hydrogen peroxide is greatly dependent on the intensity of the radiation absorbed. High quantum yields (\(\gg 1\)) are only obtained at low radiation intensities. For high radiation intensities the quantum yields were between 1 and 2. Weiss attributed this to an effective termination of \(\text{OH}^*\) and \(\text{HO}_2^*\) radicals, caused by their high concentration.

Reaction (3) is known as the "Haber-Weiss reaction". It is of interest as it is the rate determining step in the photodecomposition of \(\text{H}_2\text{O}_2\). Also, the reaction leads to the removal of \(\text{HO}_2^*\) radicals, which although not as powerful an oxidising species as hydroxyl radicals (Wolfrum et al., 1994), play an important role in reactions with enzymes and membranes and are thus of interest with respect to \(\text{H}_2\text{O}_2\)-induced microbial inactivation (Lunak and Sedlak, 1992).

Wolfrum et al. (1994) predicted the steady-state concentration of hydroxyl radicals to be proportional to the UV intensity. They found experimentally that the rate of formation of hydroxyl radicals was proportional to the hydrogen peroxide concentration at concentrations of up to 0.017 w/v\% \(\text{H}_2\text{O}_2\) (=5.0 mM).

The rates obtained by previous researchers for the photodecomposition of hydrogen peroxide show marked discrepancies. This is because other chemical species present can interact with free radicals present in the \(\text{H}_2\text{O}_2\) decomposition chain. In addition, metal ions such as \(\text{Fe}^{2+}\) and \(\text{Cu}^{2+}\) are known to act as catalysts in the decomposition of hydrogen peroxide (in the absence of UV), causing the formation of free radicals. The presence of these free radicals also causes an increase in the decomposition rate of hydrogen peroxide in the presence of UV. Copper ions are the most effective catalyst of \(\text{H}_2\text{O}_2\) lysis known so
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far. The effect of copper sulphate has been detected at concentrations as low as $10^{-7}$ M (Lunak and Sedlak, 1992). In order to accurately predict the inactivation kinetics from theory one would need to know the precise mechanisms behind the microbial inactivation and the kinetics of the formation and destruction of the relevant chemical species. Such an approach is not currently possible due to the multitude of factors affecting the decomposition of H$_2$O$_2$. Therefore the rate coefficients for the reactions involved in the photodecomposition of hydrogen peroxide must be determined every time a different water source is employed. The concentrations of the intermediate free radicals may then be calculated by assuming that the free radicals reach a steady state concentration.

Bayliss and Waites (1979) tested their hypothesis that the synergistic inactivation effect obtained with UV and hydrogen peroxide was due to hydroxyl radicals by adding free radical scavengers L-cysteine and mannitol to the spore suspensions 30 minutes before treatment to allow time for them to permeate into the cells. It was found that none of the radical scavengers had any effect on disinfection performance. They concluded that if hydroxyl radicals were responsible for the synergistic effect then they must be formed at sites within the spores that the radical scavengers could not reach.

Waites et al. (1988) found that the optimum UV wavelength was different for the combined UV + H$_2$O$_2$ process to that for UV alone. For UV induced microbial inactivation, the optimum wavelength is about 260 nm, due to absorption by the nucleic acid bases in the DNA. For UV + 1.0 w/v% H$_2$O$_2$ a maximum rate of microbial inactivation was obtained with 270 nm UV (intensity not stated). Waites et al. (1988) explained the wavelength shift as being due to the absorption of UV by the H$_2$O$_2$. Whilst it is necessary for H$_2$O$_2$ to absorb UV to produce the synergistic effect, if UV is absorbed significantly in the hydrogen peroxide the average UV intensity at the cells' surface (or inside the cells) will be reduced. This both reduces the disinfection rate due to UV solely and the concentration of hydroxyl radicals at the cells' surface and inside the cells, thus reducing the inactivation rate.

Bayliss et al. (1982) investigated the mechanism of microbial damage by the combined effects of UV and H$_2$O$_2$ treatment using strains of *B. subtilis* spores some of which were
deficient in two specific repair mechanisms. These repair mechanisms were capable of removing the spore photoproduct and of performing excision/resynthesis repair. The results showed that the spores' UV resistance was important in determining the spores' resistance to the combined treatment whilst the spores' resistance to hydrogen peroxide did not influence the resistance to the combined treatment. This shows that the combined treatment, like UV alone, primarily attacks the spores' DNA whereas hydrogen peroxide alone does not primarily attack DNA because the loss of excision/resynthesis repair made no difference to the spores' hydrogen peroxide resistance. However there might be other, as yet unidentified, repair mechanisms for \( \text{H}_2\text{O}_2 \)-induced DNA damage. Bayliss and Waites (1981) also demonstrated that microbial resistance to \( \text{H}_2\text{O}_2 \) and UV + \( \text{H}_2\text{O}_2 \) combined is related to the total catalase activity within cells. Changes in growth media can affect catalase synthesis and hence microbial resistance.

1.5.3 Ozone and hydrogen peroxide

1.5.3.1 Disinfection using aqueous \( \text{O}_3 \) and \( \text{H}_2\text{O}_2 \)

Wolfe et al. (1989) first gave the combination of \( \text{O}_3 + \text{H}_2\text{O}_2 \) the name 'Peroxone'. Most of the published work in this area deals with the oxidation of organic material present in surface waters and as for previously described oxidation treatments, there have been few published disinfection studies using this process. Wolfe et al. (1989) found that the use of hydrogen peroxide in combination with aqueous ozone did not change, or even slightly reduced, the inactivation level of \( \text{E.coli} \) in river water over a range of \( \text{O}_3 : \text{H}_2\text{O}_2 \) ratios. This they attributed to the formation of hydroxyl radicals which react with a wide range of compounds and therefore might well react with other organics present in the river water which ozone would not react with in any significant quantity. They also noted that the ozone residuals were lower in the perozone process and postulated that the lower ozone residual might cause a worse disinfection performance.
1.5.3.2 Mechanisms of inactivation and hydroxyl radical formation

As with aqueous ozone, the chemistry of the hydrogen peroxide-aqueous ozone system is dominated by free radical chemistry and for the reasons previously stated, a short review of free radical generation mechanisms will be given. The conjugate base of hydrogen peroxide can initiate the ozone decomposition chain by the following reaction (Glaze et al., 1987):

$$HO_2^+ + O_3 \rightarrow HO_2^- + O_3^-$$ (1)

Peyton described different products from the same reactants:

$$HO_2^- + O_3 \rightarrow OH^* + O_2^* + O_2$$ (2)

Staehelin and Hoigne (1985) stated that $O_2^*$ radicals can be formed in the reaction between the $HO_2^-$ ion and $O_3$, which is in agreement with Peyton. The other reaction mechanisms are the same as for the decomposition of aqueous ozone (see section 1.4). Once the ozone decomposition chain is started, hydroxyl radicals will be produced. Staehelin and Hoigne showed that hydrogen peroxide was formed in the decomposition of aqueous ozone and plays an important role in the formation of hydroxyl radicals.

Namba and Nakayama (1982) provided evidence that hydroxyl radicals are formed in ozone/hydrogen peroxide mixtures. Using a single $H_2O_2 : O_3$ ratio, they found that the degradation rates of 8 different alcohols (relative to that of ethanol) in the ozone/hydrogen peroxide mixture to be virtually identical to those due to hydroxyl radicals in aqueous solutions, as determined by Dorfman and Adams (1973).

There is disagreement between previous researchers about the ratio of $H_2O_2 : O_3$ needed to optimise the oxidation rate. For example, Glaze et al. (1987) found the optimum ratio to be equimolar with respect to the rate of destruction of trichloroethylene in well water. In contrast, Wolfe et al. (1989) found that hydrogen peroxide did not increase the disinfection performance of aqueous ozone in river water. The differences between results from
previous studies might possibly be explained by the fact that the studies were conducted in different waters. As the oxidation mechanisms of the peroxone process are attributed to free radicals it is likely that the concentrations of these radicals will strongly depend on the nature and the concentrations of the various promoters and scavengers present in each water. Therefore, optimum production of the hydroxyl radical appears to depend on a combination of factors: ozone dosage, the $\text{H}_2\text{O}_2 : \text{O}_3$ ratio, the contact time, and the composition of the water in which the sterilants are dissolved (Wolfe et al., 1989; Ferguson et al., 1990).

1.5.4 UV and ozone

1.5.4.1 Introduction

The combination of aqueous ozone and UV has been widely investigated for the oxidation of organic compounds in water treatment (Legrini et al., 1993). However, as for the other combined treatments considered, there have not been many published studies of the disinfection performance of the UV + O$_3$ system. One exception was Francis et al. (1988) who studied the inactivation of *E.coli* in aqueous ozone and UV. They found the inactivation kinetics to be first order with some tailing of the curve. The disinfection rate for the UV + O$_3$ system was faster than the disinfection rates obtained using UV or O$_3$ separately as sterilants (ozone concentration not stated).

1.5.4.2 Mechanisms of hydroxyl radical generation

When used together, UV and aqueous ozone produce hydroxyl radicals (Beltran et al., 1994). The decomposition mechanism of aqueous ozone in the presence of UV consists of the same pathways as for aqueous ozone alone with the addition of the following reactions:

$$O_3 + H_2O \xrightarrow{h\nu} H_2O_2 + O_2$$  \hspace{1cm} (1)
The second reaction has been shown to be much slower than the first and is considered negligible when considering the oxidation mechanisms of aqueous ozone (Beltran et al., 1994). The conjugate base of the hydrogen peroxide formed from the photolysis of aqueous ozone can then form hydroxyl radicals from the same pathways as described in section 1.4.3.

Beltran et al. (1994) showed that oxidation from the UV + O₃ process was almost solely due to oxidising radicals, with the contribution from direct ozonation even lower than in the case of using aqueous ozone solely. Denis et al. (1992) showed that oxidising radicals were formed in the UV + O₃ process. The oxidation rate increased with aqueous ozone concentration up to 4 mg O₃/L. These workers also showed that increasing the temperature increased the rate of oxidation.

From stoichiometry, the yield of hydroxyl radicals from the UV + H₂O₂ process is greater than that for the UV + O₃ process (1.5 moles of O₃ consumed to provide 1 mole of hydroxyl radicals compared to 0.5 moles of H₂O₂ consumed to produce 1 mole of hydroxyl radicals). However aqueous ozone absorbs UV far more strongly than hydrogen peroxide (molar extinction coefficients: O₃ = 3300 M⁻¹cm⁻¹, H₂O₂ = 20 M⁻¹cm⁻¹). Therefore for the same molar concentration of H₂O₂ and O₃ exposed to UV, a higher concentration of hydroxyl radicals will be formed in aqueous ozone (Glaze et al., 1987). Theoretically this makes aqueous ozone a more effective disinfectant than hydrogen peroxide of the same molar concentration when combined with UV.

As was the case for aqueous ozone, it is useful to know the degradation rate of aqueous ozone in the presence of UV so that one can apply doses of aqueous ozone suitable to maintain the required aqueous ozone concentration and exposure time. No practical equations for the rate of photolytic decomposition of ozone are currently available.
1.6 Aims and Objectives

The preceding review of the literature provided evidence that the inactivation of microorganisms in liquid media by UV and, to a lesser extent, advanced oxidation processes, had been extensively studied. However, relatively little work had been done on microbial inactivation on the surfaces of solid objects. In particular, the literature highlighted a deficiency in any coherent data which could be used to accurately predict the rates of inactivation of micro-organisms on surfaces for the purposes of evaluating these technologies for applications in such fields as food processing and the disinfection of medical items.

The well-documented synergistic disinfective action of UV in combination with hydrogen peroxide merited particular attention as it could offer operating advantages such as reduced treatment times. In considering how best to apply liquid disinfectants to surfaces, attention focused on sprays. The application of liquids in the form of sprays represents one method of obtaining even coverage of the surfaces of an object. Indeed, one such combination, UV and hydrogen peroxide sprays, has been patented as a disinfection treatment for the cardboard-based material from which drinks cartons are fabricated (Peel and Waites, 1983). A refinement of this type of technology is the electrostatic charging of liquid sprays to obtain enhanced surface coverage whilst minimising liquid consumption. Charged sprays have demonstrated definite advantages for car body painting and crop spraying (Bailey, 1986) and such an approach could be useful in controlling residual levels of the disinfectants, an important consideration for foodstuffs.

On the basis of these considerations, the principal objectives of this research programme were formulated thus :-

- To devise methods for studying the efficacy of UV either alone or in combination with aqueous hydrogen peroxide and ozone for disinfecting the surfaces of solid
objects.

- To obtain data on the rates of inactivation of a model organism by these methods and to apply appropriate kinetic expressions to the data.

- To establish whether the electrostatic charging of liquid sprays resulted in enhanced surface coverage and improved disinfection kinetics.

- To investigate the effects on microbial survival of disinfecting surfaces having different surface characteristics or 'micro-topographies'.

- To devise mathematical models which would permit the UV intensities on the surfaces of objects of regular geometry placed in a UV field to be predicted.

- To combine model predictions with experimentally determined inactivation data to enable an evaluation of a hypothetical process for the disinfection of objects by UV in combination with spray disinfection.
2. METHODS AND APPARATUS

2.1 Micro-organisms

2.1.1 Source

*E.coli* MGa was isolated from Mogden Sewage Treatment Works (near Kew, Surrey) and is further described by Shama (1992). *Micrococcus luteus* (ATCC 4698, NCIMB 9278), and *Bacillus subtilis* (ATCC 6633, NCIMB 8054) were purchased from the National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen.

2.1.2 Cultivation of organisms

All three organisms used were cultivated using similar techniques. Stock cultures were prepared from freeze-dried ampoules by aseptically transferring culture to 250 ml Erlenmeyer flasks which contained 100 ml Nutrient Broth (Oxoid) using a flamed inoculation loop. One loopful of organisms was used to inoculate the nutrient broth. The Erlenmeyer flasks were incubated at 30°C in a rotary incubator at 200 rpm until the organisms had reached the stationary phase in their growth cycle. This was determined by the measurement of the absorbance at 600 nm of samples using a spectrophotometer (Shimadzu UV-1201 UV-VIS Spectrophotometer, Shimadzu Europa, Duisburg, Germany). Samples (1 ml) were taken aseptically from the flasks using a micropipette (Gilson) and transferred to cuvettes suitable for use in the spectrophotometer. The stationary phase was deemed to have been reached when there was no further increase in the absorbance at 600 nm. The incubation periods required for the three organisms to reach the stationary phase were: *E.coli* MGa - 12 hours, *M.luteus* (ATCC 4698, NCIMB 9278) - 35 hours, *B.subtilis* (ATCC 6633, NCIMB 8054) - 24 hours. Growth curves for these micro-organisms are shown in Figure 2.1. The 3 micro-organisms used were stored as cultures on Nutrient Agar slopes in a refrigerator at 4°C.
FIGURE 2.1  Microbial growth curves
2.1.3 *B. subtilis* spore production

(a) Introduction

Chang *et al.* (1985) and Bayliss *et al.* (1981) demonstrated that the UV resistance of *B. subtilis* spores depended on the growth medium in which the cells were grown and also the degree to which they were heat-shocked (Toledo *et al.*, 1973). Therefore it was critically important to use a standard method to generate *B. subtilis* spores. The method used for the cultivation of *B. subtilis* spores was based on the approach of Harnulv and Snygg (1972).

(b) Procedure

An Erlenmeyer flask containing 100 ml Nutrient Broth (Oxoid Ltd., Basingstoke, Hants.) was inoculated with one loopful of *B. subtilis* from a Nutrient Agar slope. The Erlenmeyer flask was incubated at 30 °C for 24 hours in a rotary incubator at 200 rpm. The resulting *B. subtilis* culture was then used to inoculate the Sporulation Agar (SA) plates (Harnulv and Snygg, 1972):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth (Oxoid)</td>
<td>0.8</td>
</tr>
<tr>
<td>Yeast extract (Oxoid)</td>
<td>0.4</td>
</tr>
<tr>
<td>MnCl$_2$.4 H$_2$O (BDH)</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar technical grade 3 (Oxoid)</td>
<td>2.0</td>
</tr>
<tr>
<td>adjusted to pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

SA plates were inoculated with 0.2 ml of *B. subtilis* culture which was spread over the plates using a flamed glass spreader. The plates were incubated for 14 days at 30 °C.

After 14 days incubation the growth of the *B. subtilis* was confluent. Harnulv and Snygg found that over 95% of the *B. subtilis* were in the form of spores after 3 days of incubation. *B. subtilis* spores were harvested from sporulation plates by pipetting 5 ml sterile distilled water onto the surface of the plate and scraping the surface with a flamed inoculation loop.
in order to detach the spores. The resulting suspension was pipetted into sterile Universal bottles and then immediately washed in order to remove small pieces of agar. The *B. subtilis* spore suspension was transferred to sterile centrifuge tubes and centrifuged at 8000 g for 20 minutes. The supernatant was poured off and 10 ml sterile distilled water added. The pellet was resuspended by vigorous mixing using a vibratory mixer (*Whirlimixer*, Fisons Scientific Apparatus, Loughborough, Leics.). This procedure was repeated a further 2 times.

In order to inactivate any vegetative cells that might still have been present, the *B. subtilis* spore suspension was subjected to a 'heat shock'. Universal bottles containing the spore suspension were placed in a water bath at ambient temperature. The temperature of the water bath was then raised to 70 °C (typically this took approximately 30 minutes) and the spore suspension kept at that temperature for 30 minutes. The Universals were then removed from the water bath and transferred to a refrigerator at 4 °C where they were kept until required.

**2.1.4 Determination of UV resistance of spores**

In order to eliminate variability, batches of spore stock were made in sufficient quantities to last for several months experimental work. Tests were carried out to determine the UV resistance of spore stocks as they were produced. This was done by coating Grade 2 filter paper (Whatman) with spores, as described in section 2.2, and exposing to UV. The UV source used was the high intensity lamp described in section 2.3.2.

The disinfection results obtained with Grade 2 filter paper were plotted onto a graph including all previous disinfection studies for *B. subtilis* spores on this surface. If the disinfection results obtained lay within the range of results previously determined then the spore batch was deemed suitable for use. An example is shown in Figure 2.2 where the disinfection data for a new spore batch, created on 29/11/94, was compared to that obtained from previous spore batches. This new spore batch was deemed suitable for use.
FIGURE 2.2 *B. subtilis* spores on Grade 2 filter paper exposed to UV using the high intensity lamp
2.2 Coating of surfaces

2.2.1 Introduction

The principle objective of the work described here was to study the performance of a spray sterilisation chamber when test objects of different geometries were placed within it. To assess the level of disinfection obtained it was necessary to know the number of viable organisms on the object before and after treatment. As the test objects could not be directly coated with micro-organisms, test surfaces were coated with micro-organisms and attached to the objects. The best results were obtained by either filtering spore suspensions through membranes or by allowing spore suspensions to dry directly onto surfaces, e.g. filter paper or aluminium sheet.

2.2.2 Filter paper strips

(a) Grades of paper used

Two grades of cellulose filter paper (Whatman (UK) Ltd., Maidstone, Kent) were used in this work - Grade 2 and Grade 6 filter paper (made from cotton linters; particle retention of 8 \( \mu \)m and 3 \( \mu \)m respectively). Grade 2 filter paper had a more open structure (8 \( \mu \)m retention) than Grade 6 filter paper (3 \( \mu \)m retention). In addition, Glass microfibre filter paper (Whatman) was also used in certain experiments.

(b) Inoculation of the filter paper

Filter paper strips (3 cm x 1 cm) were cut out by hand and placed in sterile Petri dishes. Each strip was inoculated with the test organism by pipetting 40 \( \mu \)L of a microbial suspension onto the filter paper strip using a micropipette (Gilson). Eight aliquots of microbial suspension, total volume 40 \( \mu \)L, were applied over the surface area of the filter paper strip in a '4x2' pattern i.e. 4 aliquots along the 3 cm length and 2 aliquots along the 1 cm length. Once inoculated the filter paper strips were left in the Petri dishes to dry overnight and subsequently kept at ambient temperature in the dark and used the day after
inoculation.

(c) Recovery of spores / micro-organisms

In order to recover the micro-organisms from the filter paper strips it was necessary to return the spores to a suspension so that serial dilutions and platings could be performed. The filter paper strips were placed in sterile Universal bottles containing 10 ml sterile Ringers solution (Oxoid Ltd., Basingstoke, Hants.) and approximately 1 g ballotini (3 mm +/- 0.5 mm diameter, Jencons, Leighton Buzzard, Bedfordshire) and macerated by agitation using a vibratory mixer. Following this, the resulting fibrous suspension was allowed to settle for 40 seconds to leave a clear, fibre-free Ringers solution which yielded a full recovery of the spores from the filter paper strip. Supernatant was removed and subsequently used for serial dilutions and/or plating.

2.2.3 Membranes

(a) Types of membranes used

Two types of membranes were used in this work, both having 0.2 μm pore size - cellulose nitrate membranes (Whatman) and Anodisc membranes (Whatman). Anodisc membranes retain a smaller electrostatic charge on their surfaces than cellulose nitrate membranes, and have a regular, ‘honeycomb’ appearance when viewed under a scanning electron microscope. The Anodisc membrane is constructed of a patented inorganic material, ANOPORE™.

(b) Inoculation of the membranes

*B. subtilis* spore suspension (10 ml), diluted 1 in 250, was vacuum-filtered through each membrane, which was then placed in a sterile Petri dish and left to dry overnight in a dark cupboard at ambient temperature. Dilution was necessary in order to make the microbial count per membrane the same as the count per filter paper strip in the technique described in section 2.2.2.
It was found that the sampling technique described above for filter paper strips (see section 2.2.2(b)) provided full recovery of *B. subtilis* spores from membranes.

### 2.2.4 Non-porous materials

*B. subtilis* spore suspension was also deposited onto the surface of ' coupons' and allowed to dry. This method was used by Wang and Toledo (1986) when investigating the disinfection performance of hydrogen peroxide vapour. Aluminium, stainless steel, and PTFE coupons were coated using this technique.

#### (a) Inoculation of the surfaces

Coupons of the required material were cut into 15 mm x 15 mm squares from virgin material. In total 0.10 ml *B. subtilis* spore suspension was pipetted onto the surface of the coupon by placing 9 aliquots of 11.1 μL each across the surface of the coupon in an evenly spaced '3 by 3' pattern. The coupons were then transferred to sterile Petri dishes and placed in a 60°C incubator for 40 minutes to dry.

#### (b) Spore recovery

Full recovery was achieved by placing the coupons in sterile Universal bottles containing 10 ml sterile Ringer's solution and mixing vigorously for 1 minute using a vibratory mixer (Fisons, Loughborough, Leics.).

### 2.2.5 Attachment to objects

Filter paper strips, membranes and coupons of non-porous materials were all attached to test objects using 3 pieces of adhesive tape. The spore-impregnated test surface was stuck to one piece of tape whilst the other two pieces of tape were used to stick the tape to the
object. The tape held the test surface firmly in position so it was possible to use it on the vertical surfaces of objects. This method enabled the test surface to be removed from the tape by the use of tweezers, thus allowing fast sampling of the test surface.

2.3 Lab-based inactivation studies

2.3.1 Introduction

A number of small-scale experiments were carried out in the laboratory where better control of key parameters, such as UV intensity, quantity of sterilant applied and exposure time could be exercised.

2.3.2 Exposure to UV

The UV lamps used were either a 6 W low intensity source (TUV 6 W, Phillips Ltd, Croyden, Surrey) or a high intensity source (UV LUX 30 W, Voltarc Tubes Inc., USA) with reflector. Test materials were placed in Petri dishes and exposed to the UV lamp at a known intensity. The intensity was measured using a UV radiometer (Model ‘UVX’, UVP Inc., Cambridge, Cambs.). Preparation and sampling of test surfaces was as described in section 2.2. Figures 2.3 and 2.4 show the arrangements for the UV irradiation of samples with both lamps.

2.3.3 Exposure to liquid sterilants

These experiments were performed with B. subtilis spore impregnated Grade 2 filter paper strips. Liquid sterilant (40 μl) was applied to each strip by a pipette in 8 aliquots, evenly distributed over the filter paper surface. In measuring exposure times, these were taken as commencing from the instant the entire filter paper strip had been wetted. The filter paper strips were sampled as previously described (see section 2.2.2).
FIGURE 2.3  High intensity UV lamp arrangement
FIGURE 2.4 Low intensity UV lamp arrangement
2.3.4 Exposure to gaseous ozone

The output from an air-fed ozoniser (EA Technology, Capenhurst, Cheshire) was passed into a sealed, 0.5 L glass reaction vessel. The volumetric flowrate was measured by a rotameter and was approximately 0.5 L/min. Ten minutes were allowed for the ozone concentration to reach steady-state, after which *B. subtilis* spore impregnated aluminium coupons (prepared and sampled according to the method described in section 2.2.4) were placed in turn in the reaction vessel (using flamed tweezers) for specified exposure times and then immediately sampled.

2.4 Assessment of microbial survival following disinfection

2.4.1 Serial dilutions

Serial dilutions were performed in Eppendorf tubes by adding 0.1 ml of the sample to 0.9 ml of Ringers solution (Oxoid) and mixing the contents for ten seconds using a vibratory mixer (Fisons).

2.4.2 Plating

Tryptone Soya Agar (TSA) (Oxoid) was used to estimate microbial survival. The Miles and Misra (1938) plating technique was used with the modification that 6 aliquots of 20 μL microbial suspension, rather than 5 aliquots of 20 μL, were placed on a single agar plate using a micropipette (Gilson). The plates were left until the agar had absorbed the microbial suspension. They were then inverted and placed in a 30°C incubator. The plates were incubated until visible microbial colonies formed. These were counted and the plates returned to the incubator. When no further increase in the number of microbial colonies occurred, this count was taken to be the final result. For *B. subtilis* spores this incubation time depended on the level of damage incurred by the spores. For undamaged spores 17 hours incubation was typically sufficient to produce countable microbial colonies. The longest incubation period required was for spores treated with 29 w/v% H₂O₂ which required a 48 hour incubation period.
2.4.3 Sampling precautions

(a) Experiments using UV

Micro-organisms possess a number of mechanisms for repairing UV-induced damage (see section 1.2.5). Post recovery procedures were standardised in order to minimise repair to damaged cells. This entailed storing samples in the dark to avoid light-induced repair mechanisms and plating samples out with the minimum of delay to avoid light-independent ('dark') repair from occurring.

(b) Experiments using hydrogen peroxide

In order to exercise careful control over exposure times to $\text{H}_2\text{O}_2$ following treatment, test surfaces were rapidly transferred to Universal bottles, containing 10 ml Ringer's solution, and shaken vigorously. This resulted in rapid dilution; at the highest concentration of $\text{H}_2\text{O}_2$ applied, 40 $\mu$l of 29 w/v%, this procedure resulted in a final $\text{H}_2\text{O}_2$ concentration of 0.12 w/v%. Concentrations below 10 w/v% have been shown not to be sporicidal (Stevenson and Shafer, 1983). This was confirmed by a series of experiments.

2.5 Chamber description

The spray disinfection chamber used in this work to treat test objects is shown in Figure 2.5. The chamber was equipped with four UV sources which were situated at the top of the chamber and which formed a UV field in which objects to be treated were slowly rotated. At the lower end of the chamber was located a spray nozzle which permitted spraying with either aqueous ozone or hydrogen peroxide during irradiation.

The body of the spray disinfection chamber comprised a cylindrical piece of glass (9" diameter QVF with buttressed ends; Corning Glass Ltd., Stone, Staffs.). The glass body was sealed by means of two circular PVC plates which were bolted into place (Figure 2.6). A circular aperture was cut into the top plate in order to introduce test objects into the chamber. This aperture was sealed by means of a removable solid PVC plug. Objects to be
FIGURE 2.5  Spray sterilisation chamber
FIGURE 2.6  End plates of the spray disinfection chamber
irradiated were coupled to a stainless steel shaft which penetrated the PVC plug by means of a bayonet fitting (see Figure 2.7). The shaft was in turn connected to the shaft of a small electric motor (Citenco Ltd, Boreham Wood, Herts.). During normal operation the object was rotated at approximately 30 rpm.

The top plate was bored to permit four low pressure mercury vapour UV sources (UV LUX 30W, Voltarc Tubes Inc., USA) to be suspended in the chamber. The sources were housed in shrouds made of clear Fluorinated Ethylene Propylene (FEP) tubing (32 mm ID, 0.5 mm thickness; Adtech Ltd, Stroud, Gloucs). FEP is transparent to short wave UV and is far more robust than quartz glass which is traditionally used. The FEP tubes were sealed at their lower ends with circular push-fitted PVC caps. The FEP tubes were attached to circular aluminium caps which provided the means of suspension in the chamber. This arrangement gave a spray proof seal whilst enabling the tubes to be securely held in place (Figure 2.8).

In order to control irradiation times without interrupting power to the UV sources, brass tubular shields were placed inside the FEP tubes. ( Interruption of the power supply would have delayed experimentation as the sources required a period of several minutes in order to reach stable emission). Circumferential windows (arc 180°) were cut into the brass tubes and rings were attached to the ends of the brass tubes protruding from the FEP tubes so that the brass tubes could be conveniently and rapidly rotated, either to irradiate the object or to cease irradiation.

Initial studies revealed that the intensity of the UV field was very high and spores on test surfaces were inactivated within seconds. This arrangement would have made the collection of inactivation data over time impossible. It was therefore necessary to reduce the UV intensity. This was done by introducing stainless steel meshes (Plain Dutch Weave, nominal aperture 0.125 mm; Incamesh Filtration, Warrington, Cheshire) inside the brass tubes.

The consequences of confining the UV sources as described above was that the temperatures inside the FEP tubes rose to over 100°C, whilst the chamber temperature reached 50 °C. The emission of the UV lamps would have been considerably reduced at such temperatures. Also, as it was intended to investigate disinfection performance at near ambient temperatures, such temperatures in the chamber could not be tolerated. In order to dissipate the heat generated, compressed air was introduced into the FEP tubes via ¼"
FIGURE 2.7 Method of attachment of test object to rotating shaft
FIGURE 2.8 UV lamp holder for spray sterilisation chamber
brass tubing. These tubes ran alongside the UV sources to which they were attached by means of fine wire. The same air flowrate was used in all the disinfection experiments, which was controlled by setting the air pressure on the feed line to 2.0 bar. This air flowrate was sufficient to maintain temperatures of 40°C inside the FEP tubes. Moreover the chamber temperature remained relatively stable, increasing by under 5°C after one hour of operation.

Three test objects were made for use in the spray sterilisation chamber. These were a cylinder (hollow from the bottom to 1 cm below the top) and a disc-shaped object, both made from PVC, and a hollow cylinder constructed from stainless steel. These objects are depicted in Figure 2.9.

2 air-atomising nozzles were used in the spray chamber. The first nozzle used was a BETE 1/4 XA series siphon/gravity feed air-atomising nozzle (Lurmark, Longstanton, Cambridge). This nozzle produced a venturi action which provided a pressure difference sufficient to convey liquid up into the nozzle body where the liquid and compressed air were mixed internally to produce an atomised spray. The nozzle was made entirely from 303 grade stainless steel. The nozzle comprised separate air and fluid caps. (Table 2.1 shows manufacturer's data for the nozzle). An identical nozzle constructed from a non-metallic, oxidant-resistant material was also employed in this study. This nozzle was a 1/4” BSP siphon/gravity feed air-atomising nozzle with the same spray characteristics as the nozzle previously described (Spraying Systems Co., Godalming, Surrey) (Spray combination no. SU1A) and was manufactured from perspex.

The nozzles produced full cone spray patterns. When operating in the spray chamber the cone spray pattern only extended for 3-5 cm from the nozzle before the effect of eddies of air in the confined volume of the spray chamber destroyed the pattern, producing a chaotic, turbulent spray pattern.

In order to gain access to the spray nozzle without dismantling the spray chamber, the nozzles were located on mountings that could be inserted into the centre of the bottom end-plate. The removable seal on which the perspex nozzle was mounted is shown in Figure 2.10.
Disc-shaped object

PVC cylinder

Hollow stainless steel cylinder

FIGURE 2.9 Test objects for use in the spray sterilisation chamber
Spray combination | Air pressure / bar | Air capacity / l min⁻¹ | Liquid capacity / l hour⁻¹ at 10 cm siphon height | .. at 20 cm siphon height | .. at 30 cm siphon height | .. at 60 cm siphon height
---|---|---|---|---|---|---
SR050 | 0.7 | 11.3 | 0.87 | 0.68 | 0.53 | 
SR050 | 1.5 | 17.0 | 1.3 | 1.2 | 1.1 | 0.62 | 
SR050 | 3.0 | 28 | 1.5 | 1.4 | 1.3 | 1.1 |

Note: Perspex nozzle spray characteristics identical to those of the SR050 combination.

Spray angle for nozzle SR050 = 18°

**TABLE 2.1** Stainless steel air-atomising nozzle characteristics
FIGURE 2.10 Removable mounting for perspex spray nozzle
Electrostatically-charged sprays were generated by using the stainless steel air-atomising nozzle connected to a 30 kV power supply (Alpha Series; Brandenburg Ltd., Thorton Heath, Surrey) (Figure 2.11).

2.6 Inactivation studies in the chamber

2.6.1 Exposure to UV

Prior to irradiation, the 4 UV lamps in the chamber were allowed 15 minutes to reach a temperature of approximately 40°C to ensure constancy of power output during the course of the experiment. When the UV lamps had reached the required temperature, the test object was placed in the chamber using the methods described in section 2.5.

The object was irradiated by rotating the brass tubes through 180° thus exposing the object to the output from the UV lamps. Either 2 or 4 lamps were used. Two lamps were used when a lower UV intensity was required, as in the case when UV was combined with 1.0 w/v% H₂O₂. At the end of the required exposure period the brass tubes were rotated such that no UV from the lamps could reach the object.

2.6.2 Exposure to sterilant sprays

Objects were exposed to sprays in the chamber by attaching them to the central shaft passing through the top plate as described in section 2.5. The vertical position of the object in the spray chamber could be adjusted by altering the position of the motor. A vessel containing the liquid sterilant was placed underneath the chamber at a known siphon height and the liquid feed line to the air atomising nozzle was placed in the liquid sterilant. The feed air pressure, the siphon height and the measured spray time duration were recorded.

The spray duration time was measured from the time that the spray issued from the nozzle and not from when the air supply to the nozzle was turned on.

In the absence of UV, the exposure time to sterilant was taken as the duration from which the spray ceased to when the test surface was sampled; access to the test surface typically
FIGURE 2.11  Electrostatic spray nozzle mounting
took 40 seconds. When using UV in combination with H₂O₂ sprays which were not sporicidal (e.g. 1.0 w/v% H₂O₂), the exposure time was taken to be the time that the surface was exposed to UV.

When an object was to be exposed to a combination of liquid sterilant and UV, the object was sprayed first with the UV sources shielded. Once the object was coated with spray, the brass tubes in the chamber were rotated in order to expose the object to the desired UV intensity. This procedure was developed because the inactivation rate due to combined treatments was rapid. This also allowed the test surface to be fully coated before irradiation.

Only one test surface was attached to the solid objects at any one time. By conducting a series of experiments for which the test surfaces were subjected to varying exposure times, it was possible to accumulate disinfection rate data.

2.6.3 UV Bioassay

UV fluences can be determined by reference to the dose-response characteristics of a particular organism. This technique has been referred to as a 'bioassay' (Qualls and Johnson, 1983).

With this information it is necessary only to establish the fraction of that organism surviving exposure to UV. The dose response curve for the organism enables the fractional survival to be ‘translated’ into an estimate of the UV fluence.

In this work, dry Grade 2 filter paper impregnated with *B. subtilis* spores were used. Seven disinfection experiments were performed to generate the dose response curve for the spores (Figure 2.12). A third-order polynomial curve was fitted to this data using a commercially available software package (Fig P, Biosoft, Cambridge, Cambs.) and its inverse function enabled UV fluence to be estimated directly once fractional survival of the spores were known.
FIGURE 2.12  *B.subtilis* spore-based UV intensity bioassay (on dry Grade 2 filter paper)
2.7 Spray Impact Tests

2.7.1 Introduction

These experiments were performed to estimate the mass of spray deposited on a surface during spraying and are referred to here as 'impact tests'. Two variations of impact tests were employed - attaching paper strips to the object and weighing them after spraying (attached filter paper method), and wiping the surface of an object with paper after spraying and then weighing the paper (absorbance method).

2.7.2 Absorbance method

2 cm x 2 cm squares of filter paper (Whatman, Grade 2) were cut out, labelled and weighed on a precision balance (Mettler AJ100, Mettler Instruments Ltd, High Wycombe, Berks) just prior to use. After spraying, the object was quickly removed and the object surface wiped with the filter paper square. The filter paper was then weighed immediately on the precision balance which was positioned next to the spray chamber. It was important to minimise the delay between spraying the object, wiping the object and weighing the filter paper because evaporation, either from the object surface or from the filter paper squares, was found to be significant.

2.7.3 Attached filter paper method

3 cm x 1 cm strips of filter paper (Whatman, Grade 2) were cut out and weighed on a precision balance (Mettler AJ100). The strips were then attached to the object by adhesive tape. After spraying the object, the strips were removed from the adhesive tape and weighed.
2.8 Production and measurement of ozone

2.8.1 Production of ozone

(a) Gaseous ozone

Two ozonisers were available for the production of gaseous ozone, made by BOC and EA Technology respectively. The feeds to these ozonisers were either compressed air or compressed oxygen (BOC). When oxygen-fed, the ozonisers produced a higher concentration of gaseous ozone. Previous researchers (Rickloff, 1987; Bader and Hoigne, 1981) have quoted figures of between 4 and 8 v/v% ozone for oxygen-fed ozonisers. When air-fed, the EA Technology ozoniser produced gaseous ozone of a concentration about 1.0 v/v% (determined using the method described in section 2.8.2).

(b) Aqueous ozone

Aqueous ozone was produced by bubbling gaseous ozone produced from the ozoniser through distilled water. The experimental arrangement for the production of aqueous ozone is shown in Figure 2.13. In order to obtain a saturated concentration of aqueous ozone (40 mg/L) it was necessary to use an oxygen feed to the ozoniser. When an air feed was used the highest aqueous ozone concentration that could be obtained in distilled water at 1-2 °C was about 7 mg/L. A number of precautions were necessary to ensure consistency of production. First, prior to being exposed to ozone gas, a 200 ml charge of distilled water was placed in a 250 ml capacity Dreschel bottle and stored in a refrigerator at 4°C for one hour. The Dreschel bottle was then placed in a beaker filled with ice and then gaseous ozone was bubbled through it for one hour. This was sufficient to produce a saturated concentration of aqueous ozone. The Dreschel bottle, still in the beaker filled with ice, was transferred to the refrigerator at 4°C until required. When aqueous ozone was needed, the required volume was pipetted into a chilled Universal bottle. The Dreschel bottle was then sealed and the beaker replaced in the refrigerator.

Gaseous ozone emerging from the gas outlet of the Dreschel bottle was disposed of by bubbling it through a solution of potassium iodide. As a further precaution the equipment for aqueous ozone production was placed inside a fume cupboard.
FIGURE 2.13 Production of aqueous ozone
2.8.2 Measurement of gaseous ozone concentration

Gaseous ozone concentration was determined using the method described by the APHA Intersociety Committee (Anon., 1977). This iodometric method involved reaction between potassium iodide and ozone. The ozone gas was bubbled into an alkaline potassium iodide solution at a known flowrate for a certain period of time. The resulting solution was neutralised with phosphoric acid which allowed the liberation of iodine into solution, producing a yellow colour. The absorbance at 352 nm of this solution was measured using a spectrophotometer (Shimadzu UV-1201). The ozone concentration in the solution was calculated from this result using an experimentally-determined calibration graph (see Anon. (1977) for details). As the volume of gas sampled was known, the gaseous ozone concentration could be calculated.

2.8.3 Measurement of aqueous ozone concentration

The method used was that described by Bader and Hoigne (1981). (All reagents used were purchased from Fisons Scientific Apparatus, Loughborough, Leics.). Approximately 30 ml of 0.4 M sulphuric acid was poured into a 50 ml volumetric flask. Five ml of aqueous ozone was pipetted into the volumetric flask and the volume made up to 50 ml with 0.4 M sulphuric acid. The contents of the volumetric flask were poured into a 200 ml beaker. Five ml of 0.4 M potassium iodide solution was added to this mixture. The ozone reacted with the potassium iodide to liberate iodine into solution, producing a yellow colour. Five ml of a 1.0 w/v% starch solution was added, producing a blue-coloured starch-iodine complex. Sodium thiosulphate solution was titrated against this solution. The end point was assessed to be when the solution became clear. Sodium thiosulphate solution (10⁻³ M) was used for determining aqueous ozone concentrations of about 40 mg/L. Sodium thiosulphate solution (10⁻⁴ M) was used for aqueous ozone concentrations of about 7 mg/L.

The aqueous ozone concentration was calculated using the relationships that 1 mole of ozone liberates 0.65 moles of I₂ (Anon., 1977) and that 2 moles of sodium thiosulphate react with 1 mole of I₂.
3. EXPERIMENTAL DISINFECTION STUDIES

3.1 Introduction

In this Chapter are reported experiments in which the disinfection performance of UV, H₂O₂ and ozone, either alone or in combination, were investigated using *B. subtilis* spores on a variety of test surfaces. Exposure of the test surfaces to the disinfection agents were conducted using both the spray chamber and bench top mounted UV sources.

The effects of varying the intensity of UV light and of H₂O₂ and aqueous ozone concentrations were investigated in order to find combinations of these disinfectants which gave synergistic disinfection levels. Kinetic expressions were fitted to the experimentally obtained disinfection data in order to develop equations which could subsequently be used to predict disinfection performance for larger scale spray disinfection chambers.

3.1.1 Inactivation Kinetics

The quantitative treatment of disinfection data was reviewed in Chapter 1 but it might prove helpful to briefly reiterate the main features here.

First-order kinetics represent the simplest form of kinetic expression applicable to disinfection data. These kinetics are infrequently observed for UV inactivation and are typical only for viruses whose sensitive material is single-stranded DNA or RNA. The interaction of other factors can lead to pseudo first-order kinetics being observed (Harm, 1980h).

First-order disinfection kinetics are described by the equation:
In the work described below, first order kinetics were fitted to the disinfection data using conventional linear regression techniques.

Also discussed earlier were multi-target and multi-hit kinetics. In the former, the cell is visualised as containing multiple targets which must all be struck by UV photons for cell death to occur whereas in the latter, the cell is seen as containing a single susceptible target which must be struck a certain number of times for death to ensue. Multi-target kinetics were chosen for use in this study in preference to multi-hit kinetics because the former are more easily fitted to experimental data and also because they have successfully been applied to UV-induced inactivation of *B. subtilis* spores (Sugawara et al., 1981).

Multi-target kinetics are described by the equation:

\[
\left( \frac{N}{N_0} \right) = 1 - (1 - e^{-kF})^n
\]  

(3.2)

where  
\[ \begin{align*}  
N &= \text{microbial concentration} \\
N_0 &= \text{initial microbial concentration} \\
k &= \text{inactivation rate constant} \\
F &= \text{UV fluence} \\
n &= \text{number of targets}  
\end{align*} \]
Equation (3.2) describes a 'shouldered first-order' disinfection curve of the type first described in section 1.1.2. At low microbial survival, equation (3.2) can be simplified to:

$$\left( \frac{N}{N_0} \right) \approx ne^{-kF} \quad (3.2a)$$

Multi-target kinetics were fitted to experimental data by the method described by Harm (1980c). On a plot of $\log_{10}(N / N_0)$ vs. $F$, the intercept of the linear asymptote on the $\log_{10}(N / N_0)$ axis will be $\log_{10} n$ and the gradient will be $-0.434k$. In certain cases a degree of judgement may have to be applied in determining precisely where to delineate the first order part of the curve, but once that has been achieved it is a matter of applying linear regression techniques to obtain the gradient.

### 3.2 UV Irradiation

#### 3.2.1 Results

Results obtained during the UV inactivation of *B. subtilis* spores in distilled water are shown in Figure 3.1. The data shown represents results from six separate experiments conducted over a two year period. The data displays a shouldered first-order response with 'tailing' at higher UV fluences. Although partly occluded by data points, the 'shoulder' itself is not particularly prominent. Notwithstanding, multi-target kinetics were deemed to be appropriate and were fitted to the data obtained at UV fluences below $900 \text{ Jm}^{-2}$ and the parameters are displayed in Table 3.1.

Figure 3.2 shows the inactivation data obtained on coupons of aluminium, stainless steel and PTFE. The inactivation data obtained was similar for each of these surfaces.
FIGURE 3.1 Exposure of *B. subtilis* spores suspended in distilled water to UV
FIGURE 3.2 Exposure of *B. subtilis* spores on impervious surfaces to UV
First order kinetics were fitted to the early data (i.e. for fluences less than 500 Jm\(^{-2}\)) using linear regression and the rate constants are shown in Table 3.2.

The disinfection data obtained for aqueous spore suspensions filtered through Anodisc membranes and subsequently irradiated with UV are shown in Figure 3.3. First order kinetics were fitted to the data obtained at fluences below 400 Jm\(^{-2}\) and the rate constant is shown in Table 3.2. Although the membrane data displays a greater degree of scatter (particularly at high fluences) than that obtained for spores in aqueous suspension, comparison of the rate constants (Tables 3.1 and 3.2) reveals that the spores were inactivated at a faster rate when deposited on the membranes.

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>INACTIVATION RATE CONSTANT (Jm(^{-2}))(^{-1})</th>
<th>EXPONENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1.65 x 10(^{-2})</td>
<td>2.65</td>
</tr>
<tr>
<td>Wet Grade 2 filter paper</td>
<td>3.22 x 10(^{-2})</td>
<td>2.00</td>
</tr>
<tr>
<td>Wet Grade 6 filter paper</td>
<td>9.34 x 10(^{-3})</td>
<td>2.00</td>
</tr>
</tbody>
</table>

**TABLE 3.1** Multi-target kinetic constants for *B.subtilis* spore inactivation by UV (see Appendix 3 for standard deviations)

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>INACTIVATION RATE CONSTANT (Jm(^{-2}))(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass microfibre filter paper</td>
<td>1.10 x 10(^{-3})</td>
</tr>
<tr>
<td>Impervious materials (PTFE, aluminium and stainless steel)</td>
<td>2.28 x 10(^{-2})</td>
</tr>
<tr>
<td>Anodisc membranes</td>
<td>2.13 x 10(^{-2})</td>
</tr>
<tr>
<td>Dry Grade 2 filter paper</td>
<td>4.79 x 10(^{-3})</td>
</tr>
<tr>
<td>Dry Grade 6 filter paper</td>
<td>8.77 x 10(^{-4})</td>
</tr>
</tbody>
</table>

**TABLE 3.2** First-order kinetic constants for *B.subtilis* spore inactivation by UV (see Appendix 3 for standard deviations)

Figure 3.4 shows spore inactivation on dry Grade 2 filter and dry Grade 6 filter paper and a comparison of first order rate constants (Table 3.2) reveals that a slower rate of inactivation was obtained on the Grade 6 filter paper strips.
FIGURE 3.3 Exposure of *B. subtilis* spores on Anodisc membranes to UV
FIGURE 3.4 Exposure of *B. subtilis* spores on dry Grade 2 and dry Grade 6 filter papers to UV
Data showing the inactivation of *B. subtilis* spores on Glass Microfibre filter paper, when exposed to UV light using the high intensity UV source, are presented in Figure 3.5. This data displays considerable scatter but first order kinetics were applied in order to obtain some quantitative measure of the disinfection rate for comparison with the other materials (see Table 3.2). Overall, a much lower level of spore inactivation was achieved at fluences identical to those used for spores in aqueous suspension.

Scanning electron microscope (SEM) images were obtained for Anodisc membranes, Grade 2 filter paper, and glass microfibre filter paper coated with *B. subtilis* spores, and are shown in Figures 3.6 - 3.8. The higher magnification photographs (a) reveal the spores in relation to the different surface features whilst the low magnification photographs (b) enable comparisons of surface structure to be made.

Figure 3.9 shows inactivation data obtained using Grade 2 filter paper strips, wetted with 40 μl distilled water prior to irradiation. Also included are the results obtained with dry filter paper for comparison. The results clearly show that a greater rate of inactivation was obtained in filter paper strips when they were wetted with distilled water prior to irradiation. This result was unexpected and is commented upon in the Discussion. The curve fitted to the inactivation data for wet Grade 2 filter paper is described by multi-target kinetics and the kinetic parameters are shown in Table 3.1.

An increase in the disinfection rate (relative to dry filter paper) was also observed when Grade 6 filter paper strips were wetted with 40 μl of distilled water prior to UV irradiation. This is shown in Figure 3.10 which also shows the results obtained with dry Grade 6 filter paper. Multi-target kinetics were applied to the inactivation data for wet Grade 6 filter paper strips and the kinetic parameters are displayed in Table 3.1.

### 3.2.2 Discussion

*B. subtilis* (ATCC 6633) spore inactivation in water has been previously investigated and the results obtained here show the same shape of inactivation curve as that
FIGURE 3.5 Exposure of *B. subtilis* spores on glass microfibre filter paper to UV
FIGURE 3.6  *B. subtilis* spores on Anodisc membranes
(a) High magnification

(b) Low magnification

FIGURE 3.7  *B.subtilis* spores on Grade 2 filter paper
FIGURE 3.8  *B. subtilis* spores on Glass microfibre filter paper
FIGURE 3.9  Exposure of *B. subtilis* spores to UV on wet and dry Grade 2 filter paper
FIGURE 3.10 Exposure of *B. subtilis* spores to UV on wet and dry Grade 6 filter paper
obtained by Qualls and Johnson (1983), Chang et al. (1985) and Sommer and Cabaj (1993). Multi-target kinetics were applied to the experimental data from these studies and the kinetic parameters obtained are displayed in Table 3.3 along with the kinetic parameters obtained in this work. The rate constants (k) are comparable in all the studies and vary from $1.15 \times 10^2$ to $3.85 \times 10^2$ ($Jm^{-2}r l$. The principal difference was in the value of the exponent (n); the value for the results obtained here being the lowest of all the comparable previous studies. One possible cause might lie in the method of generating the spores; all of the previous workers employed different methods to that employed here and Bayliss et al. (1981) have shown that method of cultivation can affect the UV resistance of the spores. Additionally, when comparing n values between disinfection curves, it should be remembered that n is based on a logarithmic scale - i.e. $\log_{10} n$ is the intercept of the LOG axis of the semi-log disinfection graph so small changes in the intercept can produce large changes in the n value obtained.

Spore inactivation on coupons of aluminium, stainless steel and PTFE (Figure 3.2) seemed not to be affected by the specific nature of the material and it seemed logical to treat the data from all three materials in the same way. They are referred to as ‘impervious materials’ on subsequent figure legends. Disinfection rates on the Anodisc membranes are comparable to those obtained with spores on the impervious materials. Figure 3.6 reveals the spores to be present singly and in small clusters on the surface of an Anodisc membrane of $2\mu m$ pore size. Filtration of the spore suspension through the membrane proved to be an effective way of coating the membrane with spores.

<table>
<thead>
<tr>
<th>Rate constant k ($Jm^{-2}r l$)</th>
<th>Exponent n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.65 \times 10^2$</td>
<td>2.65</td>
<td>This work</td>
</tr>
<tr>
<td>$1.15 \times 10^2$</td>
<td>100</td>
<td>Chang et al. (1985)</td>
</tr>
<tr>
<td>$3.85 \times 10^2$</td>
<td>10.2</td>
<td>Qualls and Johnson (1983)</td>
</tr>
<tr>
<td>$2.14 \times 10^2$</td>
<td>6.31</td>
<td>Sommer and Cabaj (1993) - Method A</td>
</tr>
<tr>
<td>$3.50 \times 10^2$</td>
<td>6.31</td>
<td>Sommer and Cabaj (1993) - Method B</td>
</tr>
<tr>
<td>$1.77 \times 10^2$</td>
<td>4.68</td>
<td>Sommer and Cabaj (1993) - Method C</td>
</tr>
</tbody>
</table>

Table 3.3 Comparison of multi-target parameters for B.subtilis spores in aqueous suspensions irradiated with UV
Filter papers proved a useful medium with which to conduct experiments and preliminary experiments (results not shown) revealed spore recoveries approaching 100%. The inactivation rates for spores deposited on the surface of both grades 2 and 6 filter papers was lower than for the aluminium coupons and the 2μm membrane, as comparison of the parameters in Table 3.2 shows. The rate constant for the Grade 2 and 6 filter papers is 0.22 and 0.041 times less than that for the Anodisc membrane respectively. Figure 3.7 shows spores on the surface of Grade 2 filter papers. The surface topography appears relatively open so that whilst B. subtilis spores might lodge beneath the surface, sufficient UV light could still penetrate to those regions and inflict at least some inactivation. The results obtained here showed that inactivation of spores to levels below that detectable was achieved with this grade of filter paper (i.e. no viable spores were recovered).

The inactivation rate constant for spores on glass microfibre paper was comparable (1.25 times greater) to that for Grade 6 filter paper. The results show a considerable amount of scatter (Figure 3.5) and whilst precautions were taken to minimise handling of the glass microfibre pads, they were relatively compressible and it is possible that the routine treatment employed in cutting strips, depositing spores etc. may have resulted in irreproducible alterations to the surface of this material. Figure 3.8 shows the structure of the glass microfibre paper. Highly labyrinthine channels are revealed with some spores visible inside the channels. Spores present at depths below the surface would only receive a fraction of the incident UV light.

The faster disinfection rate on Grade 2 and Grade 6 filter paper strips when wetted was unexpected and has not previously been reported. This phenomenon was first discovered when conducting preliminary experiments into the effects of combined UV irradiation and peroxidation (See Section 3.4). The reasons advanced for this phenomenon can only be speculative. Filter paper fibres expand when wet and it is possible that this expansion might result in spores being moved towards the surface, alternatively the presence of water might have led to UV light being reflected further down into the sub-surface layers.
In order to facilitate further comparison of the results obtained here with spores in a variety of media, all the data were combined and are shown in Figures 3.11a and 3.11b. In order to aid clarity, the individual data points are not shown and polynomial curves have been fitted to the data. One striking feature of presenting the data in this way is that differences in the shapes of the disinfection curves are highlighted and suggests that the surface itself can influence the disinfection performance obtained.

The extent of spore survival obtained is comparable for Anodisc membranes, distilled water and impervious surfaces at disinfection levels less than 3 orders. At higher fluences, B.subtilis spores appear to have a lower resistance to UV on Anodisc membranes and on impervious surfaces at low fluences (less than 400 Jm⁻²) compared to B.subtilis spores in distilled water. On the remaining surfaces the disinfection levels were lower than in distilled water. However at higher fluences, greater disinfection of the B.subtilis spores was achieved in distilled water than on any of the other surfaces (as seen in Figures 3.11a and 3.11b).

The SEM photographs (Figures 3.6 - 3.8) revealed spores in association with a variety of surfaces, and it is possible to visualise each of the surfaces as offering different degrees of protection, or shielding, to the spores associated with any particular surface. Shielding effects due to surface irregularities have previously been reported. For example, in the use of UV to reduce the surface microbial count on beef steaks, Stermer et al. (1987) noted that substantial protection of the natural flora to incident UV occurred. Huang and Toledo (1982) investigated the inactivation of natural flora on the skin of whole fish by UV. They showed that UV was more effective in inactivating micro-organisms on the skin of smooth-surfaced fish, such as mackerel, than on rough-surfaced fish, such as mullet. If the degree of protection that a surface offers is not uniform over the whole surface then spores on the surface will receive different UV intensities at the same incident UV intensity. In such cases, changes to the shape of the disinfection curve are observed, and appear as disinfection curves for mixed populations (Harm, 1980i). This explains the range of shapes of disinfection curves observed for the same micro-organism, B.subtilis spores, when present on different surfaces but exposed to the same UV doses. The results obtained by Stermer
FIGURE 3.11 Summary of disinfection curves of *B. subtilis* spores exposed to UV

* PTFE, aluminium and stainless steel
*et al.* (1987) also showed a change in shape of the disinfection curve from agar plates to when the flora was present on beef steaks.

### 3.3 Disinfection using hydrogen peroxide

#### 3.3.1 Introduction

The experiments described below were conducted with a variety of materials in the form of coupons which bore *B. subtilis* spores on their surfaces. Application of H₂O₂ was either by pipette, for laboratory-based studies or by atomisation nozzle in the spray disinfection chamber. All experiments were performed in triplicate.

#### 3.3.2 Results

Disinfection experiments were performed in the spray chamber using 29 w/v% H₂O₂. PTFE coupons, onto which *B. subtilis* spores had been coated, were attached to the disc test object as shown in Figure 3.13 and subjected to a pulse of an atomised spray of H₂O₂ of between 10 and 30 seconds duration. The coupons were then left in the chamber for exposure times between 1 and 10 minutes before removal; Figure 3.12 shows this inactivation data. Identical experiments were conducted using *B. subtilis* spores on stainless steel coupons and the results obtained are shown in Figure 3.14. After 10 minutes of exposure to the H₂O₂, the viable *B. subtilis* spore count on these surfaces had been reduced to below the detectable limit (4.5 orders of disinfection).

Although multi-target kinetics were originally developed to describe the disinfection rate of micro-organisms exposed to UV, they were applied to the data shown in Figure 3.12 to provide a direct comparison of parameters between the disinfection data obtained for UV disinfection and that obtained for disinfection using H₂O₂. The parameters obtained are shown in Table 3.4.
FIGURE 3.12 Exposure of B.subtilis spores on PTFE coupons to 29 w/v% H$_2$O$_2$
FIGURE 3.13 Arrangement of *B. subtilis* spore impregnated Grade 2 filter paper strips on the disc and cylindrical objects for confirming experimentally-determined kinetics.
FIGURE 3.14 *B. subtilis* spores on stainless steel coupons exposed to sprays of 29w/v% H₂O₂
Table 3.4  Summary of multi-target parameters for Bacillus spores exposed to 25.8% or 29% H₂O₂

3.3.3 Discussion

The data obtained here shows that 29 w/v% H₂O₂ was an effective sporicide at room temperature and was capable of reducing surface counts rapidly after the notable initial shoulder. The results also demonstrate the suitability of the methods developed to the investigation of spray disinfection.

The disinfection data obtained with 29 w/v% H₂O₂, shown in Figures 3.12 and 3.14, was prone to a greater degree of scatter than that for UV disinfection. This was most likely attributable to the variation in area which the dried B. subtilis spore film occupied on the coupon surface. Despite attempts at standardising spore deposition and rates of drying, as fully described in section 2.2.4, visual inspection revealed significant differences. Whilst the coupons used in the experiments shown in Figures 3.12 and 3.14 were subjected to a quantity of spray sufficient to wet the entire area of the spore film, variations in spore film depth would have offered varying degrees of protection to sub-surface layer spores and caused the observed disinfection rate to vary.
Figure 3.12 shows that consistent disinfection data was obtained using PTFE coupons, whilst Figure 3.14 shows that the disinfection curve obtained on stainless steel coupons was variable. Iron is known to catalyse the generation of free radicals from $\text{H}_2\text{O}_2$ and the combination of $\text{Fe}^{2+}$ ions and $\text{H}_2\text{O}_2$ is known as 'Fenton's reagent' (Lunak and Sedlak, 1992). Disinfection rates in the presence of $\text{Fe}^{2+}$ ions are considerably enhanced and it is possible that Fenton's Reagent was formed, though not consistently, during the spray experiments reported here. Visible spots of rust were observed to have formed on the stainless steel coupons some hours following treatment with $\text{H}_2\text{O}_2$ sprays.

Table 3.4 presents results obtained in this study together with the parameters for multi-target kinetics which were fitted to the results obtained by Toledo et al. (1973). Although these workers did not obtain any disinfection data for spores of *B. subtilis* (ATCC 6633), they did obtain results using spores from other species of *Bacillus*, including a different strain of *B. subtilis*. Toledo et al. used 25.8% $\text{H}_2\text{O}_2$ at 24°C and their data were thus obtained at slightly different operating conditions to those employed here (29% $\text{H}_2\text{O}_2$ at 23°C). However these conditions were relatively close to each other to permit useful comparisons to be made.

The table shows that the first-order disinfection rates (k values) obtained are all within the same order of magnitude. However, large discrepancies are revealed in the values of the exponent n which relates directly to the size of the initial shoulder. The results that provided the closest agreement with those obtained in the course of this work are those for heat shocked *B. subtilis* var. globigii spores. Toledo et al. (1973) showed that length and severity of the heat shocking process affected the resistance of *B. subtilis* spores to $\text{H}_2\text{O}_2$. Toledo et al. employed a heat shock of 80°C for 20 minutes compared to the 70°C for 30 minutes used in this work. The n value shows that a much larger initial shoulder was found for disinfection with 29 w/v% $\text{H}_2\text{O}_2$ compared to disinfection with UV.
3.4 Combined UV and $\text{H}_2\text{O}_2$ disinfection

3.4.1 Introduction

Combined treatment with UV and $\text{H}_2\text{O}_2$ has been widely reported to bring about a synergistic disinfection effect (Bayliss and Waites, 1979, 1980, 1982). The objective of the experiments reported here was to establish whether these synergistic effects could be demonstrated to occur at the surfaces of materials. In addition to these studies, work is also presented on the related issue of the influence of UV intensity on disinfection rate/efficiency in combined treatment.

Confirmation was sought that the kinetic equations developed from laboratory-generated data could predict the disinfection performance in the spray chamber. Two sets of experiments were conducted in the spray chamber to assess the disinfection rates for the combined UV + 1.0 w/v% $\text{H}_2\text{O}_2$ disinfection process at spatial locations of known UV intensity. The concentration of $\text{H}_2\text{O}_2$ used was that claimed by Bayliss and Waites (1979a) to result in maximal synergistic inactivation. More concentrated aqueous solutions of $\text{H}_2\text{O}_2$ absorbed UV at 254 nm and resulted in a diminution of UV intensity and a concomitant decrease in lethality. The first experiment involved placing strips of Grade 2 filter paper coated with $\text{B.subtilis}$ spores onto two objects of different geometries. The locations of the filter paper strips on the objects were as shown in Figure 3.13. Forty microlitres of 1.0 w/v% $\text{H}_2\text{O}_2$ was pipetted onto the filter paper strips, the object suspended in the spray chamber and the object exposed to 10 seconds of UV irradiation from 2 of the 4 UV lamps. The filter paper strips were sampled to determine the reduction in spore viability for each filter paper strip location. The experiment was repeated a further two times. The UV intensities at the filter paper strip locations had been previously determined by using the bioassay method employing Grade 2 filter paper strips (see section 2.6.3). Equation (3.2a) was then used to predict the disinfection performance obtained with UV + 1.0 w/v $\text{H}_2\text{O}_2$ in the spray chamber at the determined UV intensities.
An additional test of whether the laboratory-determined disinfection kinetics were valid under the conditions that existed in the larger-scale spray chamber was to compare the disinfection rates obtained for spray disinfection within the spray chamber for the UV + 1.0 w/v% H₂O₂ process with those obtained on the laboratory-scale. Aluminium coupons were attached to the disc object as shown in Figure 3.13. The surviving fraction was determined 3 times for each exposure time in each experiment.

3.4.2 Results

Experiments were conducted with strips of spore-laden Grade 2 filter paper impregnated with H₂O₂, as described in section 2.3.3. The H₂O₂ solutions employed ranged in concentration from 0.1 to 5.0 w/v% at a UV intensity of 4.7 Wm⁻². The results are presented in Figure 3.15 and show that the highest levels of disinfection were achieved at concentrations of H₂O₂ in the range of 0.5 to 1.0 w/v%.

Figure 3.16 shows inactivation data for *B. subtilis* spores on Grade 2 filter paper strips exposed to UV light of different intensities (I), either alone (using wet filter paper) or in combination with 1.0 w/v% H₂O₂. The disinfection rate with UV and H₂O₂ combined was faster than for UV alone. There is some tailing of the disinfection curve for the combined sterilants at fluences over 100 Jm⁻². The results of Figure 3.16 also show that the disinfection level achieved depended on the fluence received and was independent of the UV intensity.

The kinetics of disinfection of *B. subtilis* spores by the combination of UV and H₂O₂ are adequately described by first order kinetics as Figure 3.16 shows; the rate constant (k) being 0.102 (Jm⁻²)⁻¹. 

90
FIGURE 3.15 Disinfection performance for *B. subtilis* spores on Grade 2 filter paper exposed to UV and a range of concentrations of $\text{H}_2\text{O}_2$
FIGURE 3.16 Exposure of *B. subtilis* spores on Grade 2 filter paper to UV + 1.0 w/v% H$_2$O$_2$
FIGURE 3.17 Exposure of *B. subtilis* spores on Grade 6 filter paper to UV and UV + 1.0 w/v% H₂O₂
Figure 3.17 shows inactivation data for *B. subtilis* spores in Grade 6 filter paper exposed to UV light and also to combined UV and 1.0 w/v% H₂O₂. Again, an increased disinfection rate is observed for the combined process. First order kinetics were fitted to the inactivation data for the combined process, where $k = 4.95 \times 10^{-2}\text{ (Jm}^{-2})^{-1}$.

Figure 3.18 shows inactivation data of *B. subtilis* spores deposited on aluminium coupons exposed to UV solely and UV combined with H₂O₂ in the spray sterilisation chamber. This data confirms that synergism was achieved with 1.0 w/v% H₂O₂ sprays when combined with UV in the spray chamber.

Different spray pulse durations were investigated to establish whether reducing the mass of H₂O₂ on the surface altered the disinfection rate. Figure 3.19 shows the results for 3 separate experiments using different spray pulse durations. It is seen that the disinfection level obtained was independent of the duration that the spray was applied to the coupons. Therefore the impact from a spray application of 10 seconds was sufficient to coat the coupons so that the maximum UV + 1.0 w/v% H₂O₂ disinfection rate was obtained.

Table 3.5 shows data for combined UV + 1% H₂O₂ treatment of filter paper strips in the UV chamber. Column 3 of the table represents estimates of UV intensities obtained by bioassay. The values of UV intensities thus obtained were used in equation (3.1) to enable estimates of log reductions to be made (presented in column 5). The experimentally determined disinfection levels were slightly higher than the predicted levels. However errors in the exposure time (due to the finite time taken to rotate the brass tubes covering the UV lamps) might easily have accounted for this discrepancy.
FIGURE 3.18 Exposure of *B. subtilis* spores on aluminium coupons to UV + 1.0 w/v% H₂O₂ in the spray chamber.
FIGURE 3.19 Effect of spray pulse duration on disinfection performance for *B. subtilis* spores on aluminium coupons exposed to UV + 1.0 w/v% H₂O₂ in the spray chamber
Table 3.5  Comparison of experimentally-determined disinfection levels to predicted disinfection levels from laboratory-based kinetics

<table>
<thead>
<tr>
<th>OBJECT</th>
<th>LOCATION</th>
<th>UV INTENSITY / Wm⁻² (*1)</th>
<th>EXPERIMENTALLY OBTAINED LOG REDUCTION (LOG₁₀[N/NN₄]) A</th>
<th>PREDICTED LOG REDUCTION (*2) B</th>
<th>A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISC</td>
<td>SIDE</td>
<td>2.71</td>
<td>-1.36</td>
<td>-1.21</td>
<td>1.12</td>
</tr>
<tr>
<td>DISC</td>
<td>TOP</td>
<td>4.97</td>
<td>-2.50</td>
<td>-2.21</td>
<td>1.13</td>
</tr>
<tr>
<td>CYLINDER</td>
<td>SIDE</td>
<td>3.44</td>
<td>-1.87</td>
<td>-1.53</td>
<td>1.22</td>
</tr>
<tr>
<td>CYLINDER</td>
<td>TOP</td>
<td>4.30</td>
<td>-2.12</td>
<td>-1.91</td>
<td>1.11</td>
</tr>
</tbody>
</table>

*1 Determined by bioassay (method described in section 2.6.3)

*2 for 10 seconds exposure from equation (3.2a)

3.4.3 Discussion

Figure 3.15 shows that the most effective combined disinfection performance was achieved with H₂O₂ solutions of concentrations in the range 0.5 to 1.0 w/v%. This agrees with the results of Bayliss and Waites (1979). The criterion for synergism is satisfied as the disinfection level obtained with the combined treatment is greater than the sum of the individual effects of UV (shown in Figure 3.15) and 1.0 w/v% H₂O₂ (no effect). This latter result confirms the findings of Stevenson and Shafer (1983) that 1% H₂O₂ is not sporicidal.

None of the data obtained for combined UV + 1.0 w/v% H₂O₂ treatment revealed the presence of an initial shoulder (see Figures 3.16 - 3.19) and therefore first-order disinfection kinetics could justifiably be applied. Bayliss and Waites (1979) also found first-order disinfection kinetics to be appropriate for combined UV + 1.0 w/v% H₂O₂ treatment for *B. subtilis* (NCDO 2129) spores. These workers also reported 'tailing' of
the curves at between 4 and 5 orders of reduction in spore viability, as was also displayed in the results obtained here. This phenomenon is possibly attributable to the existence of clumps of spores which would result in the spores near the centres of the these clumps receiving a lower UV intensity than spores near the outer periphery of such clumps.

Comparison of the rate constants obtained for Grade 2 and Grade 6 filter paper strips revealed disinfection rates to be higher in the former case both for UV alone and also for UV in combination with H_{2}O_{2} (Table 3.6) and this finding suggests that surface shielding effects are significant in both cases.

First order kinetics were seen to be applicable to spore-coated aluminium coupons when exposed to a combination of UV and 1.0 w/v% H_{2}O_{2} in the spray chamber (Figure 3.18) where \( k = 0.129 (\text{Jm}^{-2})^{-1} \). Bayliss and Waites (1979) investigated the use of the identical combination of treatments against spores of a number of *Bacillus* species including *B. subtilis* (NCDO 2129) and presented their disinfection data plotted against the exposure time. By using the stated UV intensity from later studies of theirs which employed an identical experimental arrangement (Bayliss and Waites, 1981), it was possible to determine the fluence at which the disinfection data had been obtained. Fitting first-order kinetics to their data for the combined process and multi-target kinetics to their data for UV disinfection, kinetic parameters were obtained for their results and are shown in Table 3.6 together with results obtained in this study. A comparison of the data obtained here with that of Bayliss and Waites (1981) was made by taking the ratio of rate constants for UV irradiation alone and for UV treatment in combination with H_{2}O_{2}. This procedure allows differences in kinetic responses of the spores in the two studies to be ignored whilst at the same time allowing some useful comparisons to be made. The ratios obtained are shown in Table 3.6, with values ranging from 3.17 to 5.66.
Table 3.6  Comparison of kinetic parameters for the UV and UV + 1.0 % H₂O₂ process on different surfaces and in distilled water (see Appendix 3 for standard deviations)

Figure 3.19 also reveals that changing the duration of the initial spray pulse (and hence the mass of spray deposited on the coupons) had no effect on disinfection performance over the range investigated. The coupons were observed to be saturated with spray droplets in each case, thus giving the same disinfection results. Therefore the spray regimes employed in this work were suitable for effective surface disinfection. (The mass of spray deposited in these experiments is estimated in Appendix 2).
3.5 Disinfection using ozone and ozone combined with UV

3.5.1 Introduction

Ozone is a powerful oxidant and has been widely used in water treatment, both alone and in combination with UV, to remove organic pollutants (Legrini et al., 1993). In contrast, relatively little work has been done on the use of aqueous ozone as a disinfectant. In this section the disinfection performance of aqueous ozone both alone and in combination with ultraviolet light on B. subtilis spores on filter paper strips is described. In these experiments, aqueous ozone (40 μL) was pipetted directly onto each strip and these were then immediately irradiated using the high intensity UV source. In addition, results from experiments in which the sporicidal activity of gaseous ozone was investigated using an enclosed 1 litre vessel in which spore-coated aluminium coupons were exposed are reported.

3.5.2 Results

Disinfection studies were initially undertaken with aqueous ozone concentrations of between 0.23 to 0.55 mg/L, these concentrations had previously been claimed to be sporicidal (Meltzer, 1993). No sporicidal effect was detected for exposure times of up to 10 minutes (results not shown). Consequently it was decided to use a significantly higher concentration of ozone. Figure 3.20 shows the results of treatment of Grade 2 filter paper strips coated with B. subtilis spores with a saturated ozone solution maintained at 2 °C. The concentration of ozone in this solution was 43.8 mg/L, as determined by the iodometric techniques described in Section 2.8. The aqueous ozone produced only minimal inactivation of B. subtilis spores within the exposure time investigated. Further, Figure 3.20 shows that no synergism was obtained between ozone and UV.
FIGURE 3.20 *B. subtilis* spores on Grade 2 filter paper exposed to UV and aqueous O₃
In order to compare the disinfection performance of aqueous and gaseous ozone, spores were deposited onto aluminium coupons and exposed to the output from an air-fed ozoniser as described in section 2.3.4 for exposure times of up to 30 minutes. The results are shown in Figure 3.21; only a modest amount of inactivation was achieved over the 30 minute exposure period.

3.5.3 Discussion

Ozone solutions did not exert any significant lethal effects against spores of B. subtilis on filter paper. Rickloff (1987) showed 10 mg/L aqueous ozone at 20°C to be sporicidal against B. subtilis spores dried onto porcelain cylinders when immersed in aqueous ozone. The absence of lethal effects might have been due to the thermal decomposition of the ozone immediately following application to the filter strips. It is known that aqueous ozone is unstable and decomposes to water, H_{2}O_{2} and dissolved oxygen (Staehelin and Hoigne, 1985). The saturated aqueous ozone that was produced did not decompose when kept in the glass Dreschel bottle in an ice bucket (see section 2.9) for up to 20 minutes after the supply of gaseous ozone had been turned off but some decomposition was evident by 50 minutes. However, smaller volumes of aqueous ozone proved to be much less stable: for example, transferring 4 mL of 7 mg/l aqueous ozone from the Dreschel bottle to a silica cuvette and observing its absorbance at 258 nm (using a UV spectrophotometer) showed the aqueous ozone concentration to be falling rapidly. Within 2 minutes the aqueous ozone concentration had fallen to below 0.05 mg/l. This was attributed to a combination of thermal decomposition and ozone mass transfer to the air. The average temperature of the filter paper strips was approximately 23°C. The decomposition of aqueous ozone in filter paper could therefore be expected to be even faster than in the silica cuvettes because of the greater surface area to volume ratio.

It is also possible that ozone was being decomposed by a non-thermal mechanism: in a protocol for the detection of aqueous ozone, Anon. (1977), cautioned against the use of sintered glass spargers, remarking that when these were used the final concentration
FIGURE 3.21 *B.subtilis* spores on aluminium coupons exposed to gaseous O₃ (1.2%v/v)
of aqueous ozone obtained was always less than in their absence. Moreover, the reason put forward for this phenomenon was attributed to unspecified "surface-catalysed decomposition".

In contrast to the results for aqueous ozone, the results for gaseous ozone proved more encouraging (Figure 3.21). Aluminium coupons were employed in this work because the surface needed to be resistant to the highly oxidising nature of ozone. The thin aluminium oxide layer that coated the aluminium coupons rendered them inert to ozone gas. Also, aluminium has not been previously reported to have catalysing properties when used in conjunction with oxidising compounds such as H$_2$O$_2$, unlike copper or iron. Ishizaki et al. (1986) also demonstrated that gaseous ozone was sporicidal to \textit{B. subtilis} spores, with disinfection levels of up to 5 orders of magnitude obtained within 1.5 hours at 95% relative humidity using a gaseous ozone concentration of 3.0 mg/L. Direct comparison with the results of Ishizaki are not possible as relative humidity was not determined in the experiments conducted here.

In practical terms the use of aqueous ozone possesses obvious advantages over the use of gaseous ozone. However the results reported here, though of limited scope, have highlighted potential difficulties in the use of aqueous ozone for treating objects at ambient temperatures.

3.6 Disinfection experiments employing electrostatically-charged sprays

3.6.1 Introduction

A series of experiments is described here in which an electric charge was applied to the spray nozzle whilst at the same time earthing the target, or object, against which the spray was directed. It was reasoned that operation in this way would result in enhanced coverage of the target as individual spray droplets would acquire charge and would
migrate towards the earthed object. This offered the prospect of improving coverage of the object and also of minimising consumption of the liquid oxidant (H₂O₂). Grade 2 filter paper strips were employed in these experiments because of their relatively high liquid absorbance compared to aluminium coupons, thereby slowing the rate of coverage of the surface. The filter paper strips were attached to the outside surface of the hollow stainless steel cylindrical object (Figure 3.22) by means of adhesive tape and the object was then suspended in the spray chamber. In this way, a direct earth connection was made to the cylinder. Sprays were generated using a stainless steel air-atomising nozzle which was connected to a power supply and charged to a negative potential of 15 kV. The spray was administered in pulses of 5 seconds duration per minute.

![Figure 3.22 Filter paper arrangement on hollow cylinder for electrostatic spraying experiments](image)

Concentrated H₂O₂ (29 w/v%) was employed in these experiments as previous work had shown that combined UV and 1.0 w/v% H₂O₂ treatment would have resulted in rapid disinfection and posed problems in sampling from the chamber. Another advantage of operating in this way was that interpretation of results would be facilitated by not having to account for UV effects.
3.6.2 Results

Experiments using a charged nozzle were conducted over a 4 day period and in all, three experiments were performed. On each occasion a control experiment was performed with the spray nozzle uncharged. Exposure of spore-laden strips to H₂O₂ in the chamber resulted in only low levels of inactivation. The results from the first experiment are shown in Figure 3.23 and show that charging the nozzle resulted in an enhanced disinfection effect. This effect was also demonstrated in the subsequent experiments (results not shown). However repetitive handling of the H₂O₂ caused the concentration to be reduced to unknown levels, thereby decreasing the disinfection performance at each repetition of the experiment.

3.6.3 Discussion

An increase in the disinfection rate using the charged nozzle is observed in the results of Figure 3.23 when using charged sprays, this infers that a greater impact of H₂O₂ spray on the filter paper strips occurred when using charged sprays. Once a surface is fully coated with H₂O₂ then further increases in the impact of spray should not affect the disinfection level. Therefore it can be concluded that the Grade 2 filter paper strips were not saturated with H₂O₂, otherwise they would have had identical disinfection curves.

Whilst the results in Figure 3.23 show that a greater deposition of spray occurred when using electrostatically-charged sprays, the data does not enable the increase in impact of the spray on the target to be determined. The ‘Impact Test’ methods (described in section 2.7) were developed to determine the mass of spray deposited on a surface and were employed in investigating electrostatically-charged sprays in further detail. The results are discussed in Appendix 2.
FIGURE 3.23 *B. subtilis* spores on Grade 2 filter paper strips exposed to electrostatically-charged sprays of 29 w/v% H₂O₂
3.7 Overall Discussion and Conclusions

The choice of materials and method of introducing the spores on to the surface of those materials were important considerations in the studies described here. Filtration media such as filter papers and membranes provided a relatively straightforward way of depositing spores onto the surface. In the former case the relatively absorbent nature of the filters permitted known volumes of spore suspension to be carefully deposited directly on to the surface. Whereas in the latter case, spore deposition was achieved by filtering a suspension of the spores through the membrane. Experimental results of generally good quality were obtained using a variety of materials of different surface characteristics in UV irradiation experiments.

Moreover, useful experimental data was obtained with filter media in experiments with H₂O₂ when the latter could be applied in discrete quantities as in the experiments conducted using the laboratory mounted UV sources. In particular, synergistic disinfection effects between H₂O₂ and UV were clearly revealed. However, the absorbent nature of filter papers was seen as undesirable in experiments involving liquid disinfecting agents such as H₂O₂ and aqueous ozone solutions in the spray chamber. It was reasoned that differential absorption of the peroxide during UV irradiation would have resulted in complex disinfection kinetics. A quest for alternative materials (such as aluminium, stainless steel and PTFE) ultimately revealed other difficulties: whilst aluminium and stainless steel could be employed under conditions such that they bore no surface charge (i.e. by earthing) even distribution of spores was never achieved. Considerations of surface charge was of particular concern in experiments in which the spray nozzle was charged but even spray droplets produced by uncharged nozzles are known to bear a charge (J.I.T. Stenhouse, Chemical Engineering Dept., Loughborough University; personal communication).

One very important finding resulting from the use of a diverse range of materials was that the materials offered varying degrees of protection from the incident UV light to
the spores associated with those surfaces. The implications of this are considered fully in Chapter 4.
4. Mathematical Modelling of Surface Shielding

4.1. Introduction

The experimental data of Chapter 3 indicated that the inactivation kinetics of \textit{B.subtilis} spores were influenced by the nature of the material on which the spores were irradiated. This was in part revealed by the variation in form of the inactivation curves (see Figures 3.1 to 3.11). With the exception of the stainless steel coupons, where it was suggested that Fenton's Reagent might have been generated, the materials chosen were relatively inert, that is, it could be assumed that spore inactivation was not affected by interactions with the constituents of the surface and that the intrinsic UV resistance of the spores remained constant. Direct visual evidence, in the form of SEM photographs, was however obtained to show that the physical structure of materials onto which spores were deposited might constitute an important factor in determining inactivation kinetics. For example, on materials such as glass microfibre filters (Figure 3.8), spores were revealed in spatial locations which appear remote from the surface and surrounded by the relatively large glass microfibres. The extent of penetration of spores into the body of the material would clearly influence the amount of radiant UV light 'seen' by the spores. Spore penetration would be intimately linked to the structure of the material and in the example cited above, the fibres can be visualised as offering shielding to the spores to an extent which depended on the precise nature of the association of any particular spore and adjacent fibres. The analysis presented in this chapter is based on the experimental data of Chapter 3 and constitutes an attempt to categorise materials which were employed as supports for the spores by quantifying the extent of protection or shielding offered to the spores. This was done by assuming the surface of each material to comprise a finite number of zones. It was further assumed that each zone provided a definable degree of shielding as represented by a unique value of a so-called 'Exposure Factor', which is defined below. In addition, the population of spores is visualised as being distributed among these zones (see Figure 4.1). The theory developed here provides a method of determining both the 'exposure factor' and the fractional distribution of the spore population among the zones, the number of which is set
(a) Distribution of spores in a matrix which provides shielding from incident UV light.

(b) Zonal model showing distribution of spores into two zones, each with a characteristic exposure factor, \( \alpha \), and each containing a fraction, \( f \), of the total microbial population.

**FIGURE 4.1** Example of the application of the zonal shielding model.
by the user. In order to achieve this, 'unshielded' inactivation data was required for the spores, that is data obtained under conditions such that the spores received no, or only negligible, shielding from the surface layers of the material.

A search through the literature revealed experimental data pertaining to the UV irradiation of beef steaks (Stermer et al., 1987) in a form which was amenable to analysis by the methods developed in this chapter and the application of the zonal shielding model to this data is also considered here.

4.2 Mathematical description

The fraction of the UV intensity incident on a surface which the micro-organisms present on that surface can receive is defined by an 'Exposure Factor', \( \alpha \), such that:

\[
\alpha = \frac{\text{UV intensity received by microorganisms}}{\text{UV intensity incident on surface}}
\]  

(4.1)

The product of the UV intensity incident on the surface, \( I_0 \), and the exposure time, \( t \), of the surface to UV defines the surface fluence, \( F_s \):

\[
F_s = I_0.t
\]

(4.2)

From equation (4.1), the UV intensity received by micro-organisms on the surface can be defined as:

\[
I = I_s \cdot \alpha
\]

(4.3)

The unshielded inactivation kinetics are fitted to a suitable function such that:

\[
\left( \frac{N}{N_0} \right) = g(F)
\]

(4.4)
Equation (4.4) can be any expression that provides a good fit to the experimental disinfection data obtained on a surface that provides no, or minimal, shielding. For example, if the unshielded disinfection data are fitted with multi-target kinetics then equation (4.4) takes the form:

\[ g(F) = 1 - (1 - e^{-kF})^n \]  

(4.4a)

In this study, relatively rapid disinfection data was obtained for spores irradiated on the surface of both aluminium and PTFE coupons and also Anodisc membranes. Therefore the disinfection kinetics developed on all of these surfaces could be used to provide the 'unshielded' disinfection kinetics. The disinfection kinetics for Anodisc membranes were chosen for use because scanning electron microscope (SEM) images were available for this surface and clearly showed the *B. subtilis* spores present only on the surface on the membrane (see Figure 3.6).

Unshielded inactivation kinetics can be applied to an area of the surface that provides surface shielding by rewriting equation (4.3) as

\[ F = F_s \cdot \alpha \]

Thus equation (4.4) becomes:

\[ \left( \frac{N}{N_0} \right) = g(F_s \cdot \alpha) \]  

(4.5)

The surface is treated as being comprised of a finite number of zones, \( n_z \), in which the exposure factor, \( \alpha \), and the fraction of the total number of micro-organisms on the whole surface present in the zone, \( f \), are specified. The \( f \) and \( \alpha \) values are parameters that are chosen to provide a good fit to the experimental data.
The following notation is introduced:

\[ f_j = \text{fraction of the total number of micro-organisms on the surface present in zone } j \]

\[ \alpha_j = \text{exposure factor in zone } j \]

The following abbreviation is introduced here:

\[ y = \left( \frac{N}{N_0} \right) \] (4.6)

To obtain the disinfection kinetics for the total surface, unshielded inactivation kinetics are applied to each zone on the surface using equation (4.5). By summing the number of viable micro-organisms present in each zone, the number of viable micro-organisms present on the surface is determined:

\[ N = \sum_{j=1}^{n_z} f_j \cdot N_j \] (4.7)

where \( \overline{N} = \text{number of viable micro-organisms per unit surface area present on the entire surface} \)

Combining (4.5) and (4.7) yields

\[ \overline{N} = \sum_{j=1}^{n_z} f_j N_0 \cdot g(F_s, \alpha_j) \]
Therefore, assuming the initial microbial concentration is constant over the whole surface,

\[
\bar{y} = \left( \frac{N}{N_0} \right) = \sum_{j=1}^{n_i} f_j \cdot g(F_i, \alpha_j)
\]  \hspace{1cm} (4.8)

From the definitions of \( f \) and \( \alpha \), equation (4.8) is subject to the following constraints:

\[
\sum_{j=1}^{n_i} f_j = 1.0
\]  \hspace{1cm} (4.9)

and

\[
1.0 \geq f_j \geq 0.0 \quad \text{for all } j
\]  \hspace{1cm} (4.10)

\[
1.0 \geq \alpha_j \geq 0.0 \quad \text{for all } j
\]

Equation (4.8) can be fitted to experimental inactivation data obtained on a surface that provides surface shielding by the selection of values for \( f \) and \( \alpha \) in each zone. This selection can be performed by applying least squares regression.

With \( n_p \) data points for the inactivation of a micro-organism on a surface providing surface shielding, the sum of the square of the errors, \( S \), can be defined as:

\[
S = \sum_{k=1}^{n_p} \left( \log_{10} \bar{y}_k - \log_{10} \bar{y}_{\exp_k} \right)^2
\]  \hspace{1cm} (4.11)

where \( \bar{y}_k = \) value of \( \bar{y} \) predicted by equation (4.8) for same value of \( F \), as at \( k \) th data point \( (F_{\alpha_k}) \)

i.e.

\[
\bar{y}_k = \sum_{j=1}^{n_i} f_j \cdot g(F_{\alpha_k}, \alpha_j)
\]
The best fit of equation (4.11) to the experimental data is found when $S$ is minimised:

$$\min S \text{ subject to equality constraint (4.9) and inequality constraints (4.10)}$$

(4.12)

Due to the presence of constraints, equation (4.12) forms a nonlinear programming problem.

4.3 Solving the model

The nonlinear programming problem (4.12) was solved using a computer program ZONESHLD which searches for a Karesh-Kuhn-Tucker point. A detailed description of the program is given in Appendix I.

A 'Karesh-Kuhn-Tucker point' is a local minimum of the 'Lagrangian function', $L$ (Bazarra et al., 1993). The Lagrangian function combines the function to be minimised together with the products of the equality and inequality constraints with their 'Lagrangian multipliers', $\lambda$ and $\mu$, such that:

$$L(x) = f(x) + \sum_{j=1}^{n_h} \lambda_j h_j(x) + \sum_{\substack{i=1 \\text{where} \ i \in J_A}}^{n_l} \mu_i g_i(x)$$

(4.13)

where $\mu \geq 0$, $x$ is the vector of parameters to be solved for, $h$ is the matrix of equality constraints, and $\mu$ is the matrix of inequality constraints. $J_A$ is the set of active inequality constraints.
At a local minimum subject to the constraints, there exist values for the multipliers such that

$$\nabla L(x) = \nabla f(x) + \sum_{j=1}^{n_h} \lambda_j \nabla h_j(x) + \sum_{i=1}^{n_i} \mu_i \nabla g_i(x) = 0$$  \hspace{1cm} (4.14)

A local minimum is found by applying the following iterative strategy:

**STEP 1.** Make all inequalities inactive and set all Lagrangian multipliers for the inequality constraints to zero.

**STEP 2.** Solve $\nabla L = 0$.

**STEP 3.** If all the inequality constraints are not violated and there are no Lagrangian multipliers for the inequality constraints that are < 0 then STOP, a Kuhn-Tucker point has been found.

**STEP 4a.** Remove the inequality constraint with the largest negative multiplier.

**STEP 4b.** Add all violated inequalities to the active set.

Applying the above equations to the case of surface shielding:

$$x = f \text{ and } \alpha \text{ in each of the } n_z \text{ zones}$$

$$f(x) = S, \text{ the sum of the square of the errors, defined in equation (4.12)}$$

$$g(x) = \text{ inequality constraints, defined in equation (4.9)}$$

$$h(x) = \text{ equality constraint, defined in equation (4.10)}$$

Step 2 of the iterative set strategy, the solution of $\nabla L = 0$, is the rate-determining step of
the process. The program ZONESHLD uses the Newton method (Bajpai et al., 1988) to find the minimum value of the Lagrangian function. The method used to complete Step 2 will be discussed in further depth in Appendix 1.

On exit from the computer program ZONESHLD, the parameters f and α in each zone were given.

4.4 Results

Figures 4.2 - 4.4 show the disinfection data previously described in Chapter 3 for respectively, dry Grade 2 filter paper, dry Grade 6 filter paper and glass microfibre filter paper, along with the curves fitted from equation (4.8). Exposure factors were calculated for B.subtilis spores on these surfaces and are shown in Table 4.1. The sum of the square of the errors, S, divided by np, the number of data points (not those at the origin), is also displayed in Table 4.1; the lower this value, the 'better' the fit of the zonal shielding model to the experimental results. The results from the zonal shielding model are also shown in histogram format in Figure 4.5.

<table>
<thead>
<tr>
<th>Surface</th>
<th>n1</th>
<th>np</th>
<th>f</th>
<th>α</th>
<th>S / np</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Grade 2 filter paper</td>
<td>2</td>
<td>26</td>
<td>0.440</td>
<td>1.00</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.560</td>
<td>0.211</td>
<td></td>
</tr>
<tr>
<td>Dry Grade 6 filter paper</td>
<td>3</td>
<td>16</td>
<td>0.426</td>
<td>0.303</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.229</td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.345</td>
<td>0.0138</td>
<td></td>
</tr>
<tr>
<td>Glass microfibre filter paper</td>
<td>3</td>
<td>19</td>
<td>0.131</td>
<td>0.637</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.721</td>
<td>0.0883</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.148</td>
<td>0.0271</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1 Exposure factors for zonal shielding model

As explained earlier, it was also possible to estimate the exposure factors on beef steaks from the results of Stermer et al. (1987) who investigated the application of UV as a disinfectant for meat. By culturing the natural microbial flora of beef on agar plates and irradiating these cultures under a UV source, they were obtaining data under conditions in
FIGURE 4.2 Application of surface shielding model to the UV disinfection results obtained on dry Grade 2 filter paper strips
FIGURE 4.3 Application of surface shielding model to UV disinfection results obtained on dry Grade 6 filter paper strips
FIGURE 4.4 Application of surface shielding model to UV disinfection results obtained on Glass microfibre filter paper
FIGURE 4.5 Exposure factors for surfaces providing surface shielding
which the microbial colonies received little or no shielding from the incident UV, i.e. the
inactivation kinetics of this microbial flora constitutes the unshielded kinetics as previously
defined (see Figure 4.6). First order kinetics were fitted to the data by regression (using the
computer program FigP, Biosoft) \( k = 0.153 \text{ (Jm}^{-2}\text{r}^{-1}) \).

Stermer et al. also presented data for the irradiation of beef steaks under the same UV
source (see Figure 4.7). The curve fitted to the data shown in Figure 4.7 was determined by
equation (4.8) and the parameters are given in Table 4.2 below. The following results were
obtained with a 3 zone model:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ZONE 1</th>
<th>ZONE 2</th>
<th>ZONE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population fraction, f</td>
<td>0.260</td>
<td>0.725</td>
<td>0.0156</td>
</tr>
<tr>
<td>Exposure factor, ( \alpha )</td>
<td>0.749</td>
<td>0.517</td>
<td>0.00352</td>
</tr>
</tbody>
</table>

Table 4.2 Results from the application of the zonal shielding model to beef steaks

4.5 Discussion

The strategy adopted using the program ZONESHLD was to increase the number of zones
until no further improvement in the solution could be made. The sum of the square of the
errors was used to quantify the quality of the fit of the zonal shielding model to the
experimental data. In certain instances it was found that increasing the number of zones
resulted in a solution in which the additional zone bore an identical value of \( \alpha \) to the
previously obtained solution, i.e. a zone had in effect been split into two zones having
identical values of the exposure factor. Such solutions offered no advantages and so were
ignored.

Three zones were required for accurate representation of the disinfection curves obtained
on the materials offering a higher level of shielding, i.e. dry Grade 6 filter paper and glass
microfibre filter paper. Also included in this category were the beef steak results of Stermer
FIGURE 4.6 ‘Unshielded’ disinfection data for beef flora (from the paper by Stermer et. al, 1987)
FIGURE 4.7 Disinfection data for beef steaks exposed to UV (from Stermer et al, 1987)
et al. (1987). Some of the exposure factors tend to zero for these surfaces which implies total protection from UV. The lowest factors were observed for beef steaks. The disinfection curve for this material tailed off at about 2 - 3 orders of disinfection (see Figure 4.7), which is reflected in the value for \( \alpha \) in the third zone (where \( \alpha = 0.00352 \)).

Initially it was reasoned that increasing the number of zones beyond 3 might offer better solutions of the zonal shielding model. However this tends to lead to the formation of zones in which the exposure factor to tends towards zero; in addition the spore population occupying these zones would be very low indeed. This created difficulties in convergence towards a solution because in general a large range of values for \( f \) and \( \alpha \) over multiple zones renders the system of equations describing the model ill-conditioned. In practice therefore, all the inactivation data obtained were described using three zones or less.

Whilst the zonal model developed here proved useful in describing inactivation data caution should be observed in interpreting the results obtained too literally. In reality, the surfaces of materials are unlikely to contain a fixed number of discrete zones each having characteristic shielding properties but rather a distribution of areas with less well defined characteristics. A further advantage of the model is that it allows the disinfection kinetics due to UV to be described by the same equations that were used for unshielded surfaces.

It is also seen that this technique can be successfully applied to data other than that obtained in this work. However, an exhaustive search through the literature revealed only one study (that of Stermer et al. (1987)) having data in a form amenable to analysis by the methods described here. One example of a study not providing data in form required is that of Huang and Toledo (1982) who investigated the use of UV as a disinfectant for the external surfaces of whole fish. They obtained disinfection data for the natural flora on the fish skin when exposed to UV but unfortunately they did not provide any data for the disinfection rate of the natural flora when exposed to UV on an unshielded surface. However they did find that the disinfection rate obtained on smooth-surfaced fish, such as mackerel, was greater than that obtained on rough-surfaced fish, such as mullet.
4.6 Conclusions

A zonal shielding model was developed to account for the protection offered to surface-associated spores from incident UV light by virtue of surface topography. Application of the model to the experimental inactivation data obtained in this work and also to the data of Stermer et al. (1987) yielded expressions which described the data well.

Application of the model to materials offering a relatively high degree of shielding from incident UV light revealed zones which displayed relatively low values of the exposure factor, $\alpha$. This was an interesting result and suggests that spores would continue to remain protected as the UV fluence was increased. Experiments were not conducted to establish whether this was indeed the case but it is clearly one area for future studies.

Another intriguing question raised is that once a particular surface has been characterised by conducting experiments with a model organism (e.g. B.subtilis spores) what is the minimum additional data that would allow predictions to be made about inactivation kinetics for different microbial species? In particular, would unshielded inactivation data for the different microbial species be sufficient? Investigations of this nature were outside the scope of the work described here but again, this could well prove a fruitful area for future work.
4.7 Notation

F \quad \text{UV fluence}
F_s \quad \text{surface fluence}
f \quad \text{fraction of the total number of micro-organisms present in a particular zone}
g(F) \quad \text{unshielded inactivation kinetics equation (see equation (4.4))}
I \quad \text{UV intensity incident on surface}
j \quad \text{zone number}
N \quad \text{number of viable micro-organisms per unit surface area}
N_0 \quad \text{initial value of } N
\bar{N} \quad \text{N for the whole surface}
n_p \quad \text{number of data points}
n_z \quad \text{number of zones}
S \quad \text{sum of the squares of the errors of the fit of equation (4.8) to the experimental data}
t \quad \text{exposure time of surface to UV}
y \quad \text{N/N}_0
\alpha \quad \text{exposure factor due to surface shielding from UV}
5. UV FIELD MODELLING

5.1 Introduction

A variety of mathematical models have been developed to predict the intensity field produced by a UV source (Alfano et al., 1986). These models are either 'incidence' or 'emission' models. Incidence models require knowledge of values of the UV intensity close to (or inside) the region in which predictions of the UV intensity are required. This data is then used to extrapolate to other spatial locations based on equations describing the emission characteristics of the UV source. Emission models, on the other hand, can predict the UV intensity using a model for the emission characteristics of the source providing that the power output from the source at the wavelength range of interest is known. Manufacturers often provide such data.

UV intensity models can be further classified according to the method that they employ to describe the emission characteristics of the source. These are 'parallel-plane emission', 'spherical emission' and 'diffuse emission'. The simplest model is the parallel-plane emission model. This model assumes that each differential volume of the source only emits light in a plane parallel to the point of emission, i.e. that it is 2-dimensional. The spherical emission model describes 3-dimensional emission. In this model, each differential volume of the UV source approximates to a point which is assumed to emit light evenly in all directions. The equation describing such emission from a point source is known as Lambert's law (Jacob and Dranoff, 1970):

\[ I(\rho) = \frac{S_p}{4\pi\rho^2} \cdot e^{-\mu\rho} \]

(5.1)

where

- \( I \) = UV intensity / Wm\(^{-2}\)
- \( S_p \) = Power output from point source / W
- \( \rho \) = distance from point source / m
- \( \mu \) = attenuation coefficient / m\(^{-1}\)
Diffuse emission models consider the emission from a UV source to be produced in every direction, that is in a diffuse way.

The physical dimensions of the source are sometimes approximated to a line (uni-dimensional) in order to simplify the form of the equation describing the emission characteristics of the source. Such models are referred to as 'line source' models. Where the source is considered to be 3 dimensional and to emit light evenly throughout the volume of the source, the model is described as an 'extense source' model (Alfano et al., 1986).

The equations previously described predict the 'point UV intensity'. This is the UV intensity at a given point in space which receives the total energy of every ray of light intersecting it. The point UV intensity is therefore applicable when considering spatial locations in gases or liquids. On a surface however, the angle of the surface relative to the emission source needs to be known in order to calculate the UV intensity at the surface. This is because the quantity of UV energy received by a surface depends on its projected area, not the actual area of the surface. The projected area divided by the actual area of a surface is known as the 'view factor' (Bennett and Myers, 1983). The intensity on a surface will be less than the point UV intensity if the surface is not directly facing the point source (an example is shown in Figure 5.1). It was not possible to integrate analytically the UV emission models when taking account of view factors and hence computer programs based on numerical methods must be written to solve them.

\[
\Phi = \int I \cos \theta \, dA
\]

\[
I = I_p \frac{A_p}{A} = I_p \cos \alpha
\]

Figure 5.1 Demonstration of view factors
The power output from the UV sources employed in the spray disinfection chamber was known from manufacturer’s data for the power output at 253.7 nm, which is the emission line from a mercury vapour source that produces the principal germicidal effect. This value was 13.1 W. As the power output was known, an emission model was employed to predict the UV intensity at a wavelength of 253.7 nm. No previously developed mathematical models were available that could have been easily applied to the case of predicting the UV intensity on objects within the spray disinfection chamber. Therefore an extensive source with spherical emission (ESSE) model was created that took the view factors into account.

### 5.2 Mathematical description

The building block of the mathematical model used here is Lambert’s law, the equation for the point UV intensity produced by a point source. Air and water do not absorb UV light of 253.7 nm wavelength and hence for modelling the UV field in the spray chamber it can be confidently stated that the attenuation coefficient, $\mu = 0$. Therefore equation (5.1) can be rewritten as:

$$I(\rho) = \frac{S}{4\pi \rho^2}$$  \hspace{1cm} (5.2)

In the spray chamber, the UV sources were located close enough to the test object that changing the distance between the UV sources and the test object by the diameter of the UV sources would provide a significant change in the UV intensity received by the test object. Therefore it was necessary to use a model of the source which took account of the finite dimensions of the source. The model used was based on the Extensive Source with Spherical Emission (ESSE) model in cylindrical polar co-ordinates (Alfano et al., 1986). This model not only took account of the finite volume of the UV source but it assumed that UV light was being emitted evenly throughout the volume along the arc length of the source.

The model assumes that the volume of the source is comprised of elements of differential
volume \( dV = r d\theta . dr . dl \). As \( d\theta . dr . dl \) all tend to zero, the differential volume tends to a point and hence the equation for a point source can be used to describe the emission characteristics of a differential volume of a UV source. By integrating over the emitting volume of the UV source, the point UV intensity at a given spatial location \((r_1,l_1,\theta_1)\) can be calculated from the following equation:

\[
I(r_1,l_1,\theta_1) = \int \int \int \frac{S_x}{4\pi \rho^2} \cdot r . d\theta . dr . dl
\]

(5.3)

where \( S_x = \frac{S}{V_L} \)

and \( \rho^2 = (r \sin \theta - r_1 \sin \theta_1)^2 + (r \cos \theta - r_1 \cos \theta_1)^2 + (l - l_1)^2 \)

Equation (5.3) can be integrated analytically in one dimension with integration in the two remaining dimensions having to be performed numerically. If equation (5.3) is integrated along the length of the source then it becomes a summation of line sources with spherical emission. This model is suitable to determine the point intensity in non-absorbing liquids or air.

To determine the UV intensity at a surface the view factors must be taken into account. The example shown in Figure 5.1 is for two dimensions. The quantity \((\cos \alpha)\) is termed a 'view factor' and is the fraction of the point UV intensity that the surface receives due to its orientation relative to the UV source. When considered in three dimensions, a second view factor must be introduced for the perpendicular plane. Hence the equation describing the UV intensity received by a differential area of surface from a point UV source is:

\[
\bar{I}(\rho) = \frac{S_x}{4\pi \rho^2} \cdot (\cos \alpha) \cdot (\cos \beta)
\]

(5.4)

Using equation (5.4) in place of equation (5.2) causes the ESSE model to take the form:
\[ \tilde{I}(r_l, l_l, \theta_l) = \int_{l=0}^{L} \int_{r=0}^{r_l} \int_{\theta=0}^{\theta_l} \frac{S_v}{4\pi \rho^2} (\cos\alpha) (\cos\beta) \, r \, d\theta \, dr \, dl \]  

\[(5.5)\]

(cos\(\alpha\)) and (cos\(\beta\)) are functions of the coordinates of the UV point sources and the point on the surface, as well as the orientation of the surface. Consequently equation (5.5) cannot be integrated analytically. It can be solved numerically by assuming that the UV source consists of a finite number of point UV sources, \(N_p\). The sum of the power output from the point UV sources equates to the power output from the UV source:

\[ N_p, S_p = S \]  

\[(5.6)\]

![Figure 5.2 Location of point sources in the UV source model](image)

The volume of the UV source can be divided into elements of equal volume using the scheme shown in Figure 5.2. The length of the UV source is divided into a number of zones, \(N_z\), of equal length. The cross-sectional area of the UV source is divided into a number of concentric circles of equal areas by a number of lines, \(N_L\), separated by an angle of \(\frac{\pi}{N_L}\). By placing a point source at the center of each finite volume element comprising the UV source, equation (5.5) can be rewritten as:
\[
\overline{I}(r_1,l_1,\theta_1) = \frac{S_j}{4\Pi} \sum_{i=1}^{N_s} \sum_{j=1}^{N_s} \sum_{k=1}^{N_s} \frac{1}{\rho^2} \cdot (\cos \alpha) \cdot (\cos \beta)
\]  

(5.7)

where \( \rho^2 = (r_1 \cos \theta_j - r_1 \cos \theta_i)^2 + (r_1 \sin \theta_j - r_1 \sin \theta_i)^2 + (l_k - l_i)^2 \)

\((\cos \alpha)\) and \((\cos \beta)\) are calculated from the known surface orientation (at which the UV intensity is being determined) to the UV point sources. \( \rho^2, (\cos \alpha), (\cos \beta) \) are calculated for every point UV source.

5.3 Verifying the mathematical models

To verify the emission form of the ESSE model developed, it was necessary to obtain UV intensity data at known spatial locations produced by unshielded UV lamps. Such data was not available from the spray chamber because the UV lamps within the spray chamber were located inside the brass tubes and meshes which significantly reduced the UV output from the UV lamps into the spray chamber. The manufacturer of the Voltarc G30 sources employed in the spray chamber quoted that a UV intensity of 120 \( \mu \text{W/cm}^2 \) (for 254 nm wavelength) should be obtained at a distance of 1 m from the UV source. The ESSE model predicts a UV intensity of 109 \( \mu \text{W/cm}^2 \) based on the manufacturer's quoted power output at 254 nm of 13.1 W. The manufacturer's estimated power output cannot be used to predict the UV intensity in the experimental spray chamber because the UV sources are located behind meshes which reduce the UV intensity. However by using the ESSE model to estimate the effective power output of the UV sources based on UV intensity measurements within the chamber, it is possible to predict the UV intensity field within the experimental chamber. In effect, this is converting the ESSE model into an incidence model.

A UV radiometer was located inside the spray sterilisation chamber using the arrangement shown in Figure 5.3. As the location of the UV radiometer was known in relation to the
UV sources in the spray chamber, it was possible to use the ESSE model to calculate the power output of the UV sources from the intensity measurements taken using the radiometer. By re-arranging equation (5.7) to calculate $S_p$ for each UV intensity measurement made with the radiometer, $I(r_i, t_i, \theta_i)$, the effective power output from the UV source can then be determined from equation (5.6) which would produce the UV intensity measured at the given spatial location. A computer program was written to perform these calculations - POWERFOR (see Appendix I). The 'apparent' power output for one UV source in the spray disinfection chamber was estimated at between 0.491W - 0.528W at 254 nm when placed inside a UV source holder. Errors in determining the precise spatial location of the radiometer sensor can account for the slight variation in the estimated 'apparent' power output from one UV source.

![Arrangement for UV intensity measurements in the spray disinfection chamber using the radiometer](image)

**Figure 5.3** Arrangement for UV intensity measurements in the spray disinfection chamber using the radiometer

In addition to employing a UV radiometer to provide UV intensity measurements within the spray chamber, the dry Grade 2 filter paper bioassay technique (see section 2.6.3) was employed to provide UV intensity measurements. As the disinfection data on which the
bioassay curve is based was produced independently from the series of experiments conducted within the spray chamber, this technique for providing UV intensity measurements was used to verify the predicted UV intensity field within the spray chamber.

Three experiments using the disc test object and the cylindrical test object were undertaken in the spray chamber using the dry Grade 2 filter paper bioassay to determine the average UV intensity over areas covered by filter paper strips on the surfaces of the objects (see section 2.6.3). The locations of these areas are shown in Figure 5.4. The UV field model was used to generate average UV intensities within these same areas and the results compared. The results of the bioassays and model predictions are shown in Table 5.1. It is seen that the intensity predicted by the model is 75-95% of the intensity determined by the bioassay. This shows reasonable agreement.

A possible source of error was the effect of the mesh. It was assumed that the mesh reduced the UV intensity by the same proportion no matter what the spatial location within the rig. However, the reduction in UV intensity will change depending upon the angle from which the mesh is viewed. For simplicity this effect has been ignored and the comparison between experimental and model results would suggest that the effect can be neglected.

5.4 Predicting UV intensity fields on surfaces

It is possible to predict the UV intensity fields for a wide range of UV lamp arrangements and object geometries. In this section are presented a small selection of results obtained from applying the ESSE model. As has been previously mentioned, each object geometry will produce a unique set of view factors at the object surface and therefore separate computer programs must be written to solve for different object geometries. Two object geometries are considered here - cylindrical and slab geometries.
### Table 5.1

Comparison of model results and bioassay results for the UV intensity in the spray chamber at different locations.

<table>
<thead>
<tr>
<th>Object (see below)</th>
<th>Location</th>
<th>Average</th>
<th>Low</th>
<th>High</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIDE</td>
<td>2.46</td>
<td>2.08</td>
<td>3.13</td>
<td>2.09</td>
</tr>
<tr>
<td>1</td>
<td>TOP</td>
<td>4.52</td>
<td>4.33</td>
<td>4.71</td>
<td>3.75</td>
</tr>
<tr>
<td>2</td>
<td>SIDE</td>
<td>3.27</td>
<td>2.71</td>
<td>3.54</td>
<td>2.48</td>
</tr>
<tr>
<td>2</td>
<td>TOP</td>
<td>4.09</td>
<td>4.00</td>
<td>4.17</td>
<td>3.85</td>
</tr>
<tr>
<td>3</td>
<td>H1</td>
<td>4.92</td>
<td>4.53</td>
<td>5.33</td>
<td>4.38</td>
</tr>
<tr>
<td>3</td>
<td>H2</td>
<td>4.83</td>
<td>4.53</td>
<td>5.00</td>
<td>4.00</td>
</tr>
<tr>
<td>3</td>
<td>H3</td>
<td>3.42</td>
<td>3.20</td>
<td>3.58</td>
<td>2.69</td>
</tr>
</tbody>
</table>

Note: Refer to figure below for object number and location.

### FIGURE 5.4

Locations of Grade 2 filter paper strips for bioassay intensity measurements.
5.4.1 Cylindrical geometry

The disinfection studies that were performed during this work were conducted on the disc test object. Therefore it was valid to consider the UV intensity fields that would be produced on a cylinder within the spray chamber.

Predictions for the UV intensity fields produced on the disc test object in its experimental location (i.e. with the top surface level with the bottom of the windows in the brass tubes and suspended from the shaft along the axis of the spray chamber - see Figure 5.5) were made using three computer programs - VOLTARC, VERT and MULTIVOL. The programs VOLTARC and VERT were employed to calculate the UV intensities produced on the top and side of a cylindrical object exposed to the output of one UV source respectively. Once the UV intensity field produced by one UV source was known, the UV intensity field produced by many UV sources could be predicted by summing the intensity fields produced from each UV source. The program MULTIVOL was used to perform the summation calculation. The listings for these computer programs are given in Appendix 1.

Figures 5.6 - 5.9 show the UV intensity fields on the disc test object in the spray chamber irradiated by either 1, 2, 3 or 4 UV sources respectively. (The UV sources were equidistant from one another). For the side intensity profile, the UV intensity around the circumference of the side of the disc at the top edge is shown. For the top surface, the UV intensity at a radial distance of 27 mm is shown. This radial distance was the location of the centre of the B.subtilis spore-coated coupons on the top surface of the disc test object during the disinfection experiments described in Chapter 3. The manufacturer’s power output of 13.1 W was used in generating these results.

The figures 5.6 - 5.9 show that increasing the number of UV sources not only increases the UV intensity produced on the disc but also creates a more even UV intensity field, particularly on the side of the disc which will be shielded from the output of some of the UV lamps at certain orientations. Figure 5.7(a) shows that the UV intensity fell to only approximately 80% of its maximum value during the disinfection experiments.
FIGURE 5.5  Object geometries in the spray disinfection chamber
FIGURE 5.6  UV intensity profiles on disc test object in the spray chamber produced by a single UV source
FIGURE 5.7 UV intensity profiles on disc test object in the spray chamber produced by 2 sources
FIGURE 5.8  UV intensity profiles on disc test object in the spray chamber produced by 3 UV sources
FIGURE 5.9  
UV intensity profiles on disc test object in the spray chamber produced by 4 UV sources
(a) Top surface

(b) Side

FIGURE 5.10  UV intensity profiles on disc test object in the spray chamber produced by 2 UV lamps
Figure 5.10 compares predicted UV intensity fields for the disc when using 2 UV sources which are located 16 cm from the centre of the disc, compared to 8 cm in the case shown in Figure 5.7. It is seen that the UV intensity is lower than the case in Figure 5.7 but the UV intensity field shows less variation for both the top and side of the object.

5.4.2 Slab geometry

No disinfection experiments were conducted with a test object of slab geometry but this geometry provided an interesting comparison to the cylindrical geometry previously described. UV intensity fields were produced for a cube of dimensions 66 mm x 66 mm x 66 mm located at the axis of the spray chamber and at a height where the centre of the cube was level with the middle of the windows in the brass tubes (see Figure 5.5). The location of the cube was chosen so that the intensity fields on the sides would be the same and those on the top and bottom of the cube would be identical. The manufacturer’s data of 13.1 W was used for the power output of the lamp.

Figures 5.11 shows the UV intensity fields produced on the sides and top/bottom of the cube when the sides of the cube are facing the UV lamps, i.e.

\[ \begin{array}{c}
\circ \\
\circ \quad \square \quad \circ \\
\circ 
\end{array} \]

The origin of the Cartesian co-ordinates is located at the centre of the cube. The highest intensities on the top and bottom surfaces were achieved at the four corners, whereas for the sides, maximum intensity was attained along a line traversing the centre.
FIGURE 5.11  UV intensity profiles on a cube in the spray chamber produced by 4 UV lamps facing the center of each side
FIGURE 5.12  UV intensity profiles on a cube in the spray chamber produced by 4 UV lamps facing the edges between the sides of the cube.
Figure 5.12 shows the UV intensity fields produced on the cube when it is rotated through 45° around its central axis, i.e.

As for the previous configuration, maximum intensities on the top and bottom surfaces of the cube were achieved at the corners, however a greater variation in intensity is apparent. In contrast to the previous configuration, the maximum intensity attained on the side faces occurred along the edges, with the maximum intensity now being at the centre.

5.5 Conclusions

The UV intensity field generated by the arrangement of sources in the spray chamber was modelled by creating an extense source with spherical emission model. This enabled the intensities on the surfaces of objects of different geometrical form suspended in the UV field to be predicted. Although the routines established permitted consideration of a wide variety of shapes, particular attention was focused on cylinder and slab geometries. The versatility of the developed models was further demonstrated by examining the effects of irradiation by either 1, 2, 3 or 4 sources and of increasing the distance between object and source.

Where practicable, verification of model predictions and radiometer and bioassay measurements were made, and these gave acceptable levels of agreement. This agreement provided justification for further developments of the model and its application as described in Chapter 6 to situations in which objects are being sprayed with hydrogen peroxide whilst undergoing UV irradiation.
5.6 Notation

\( I \) point UV intensity

\( \bar{I} \) UV intensity on a surface

\( L \) arc (emitting) length of UV source

\( N_c, N_L, N_z \) number of circles and lines dividing the UV source

\( \) cross-sectional area, and the number of lines dividing the

\( \) length of the UV source, respectively, into equi-volume

\( \) elements (see Figure 5.2)

\( r, l, \theta \) cylindrical polar coordinates

\( r_L \) radius of UV source

\( S \) Power output from UV source

\( S_P \) power output from a point UV source

\( S_V \) power output from UV source per unit emitting volume

\( V_L \) emitting volume of UV source

\( \rho \) distance from point source

\( \mu \) (Base e) attenuation coefficient for UV in a medium

\( \cos \alpha, \cos \beta \) Surface view factors
6. Combined UV-H$_2$O$_2$ Disinfection Model

6.1 Introduction

Kinetic equations describing the disinfection kinetics of *B. subtilis* spores when exposed to UV, either alone or in combination with H$_2$O$_2$, were obtained from experimental results in Chapter 3 and models describing the UV intensities produced on an object in a UV field were developed in Chapter 5. Combining these two models together could enable the degree of disinfection that could be achieved for a solid object undergoing spraying with 1% H$_2$O$_2$ in a UV field to be predicted.

A method of achieving this combination is described in this chapter and is illustrated with reference to a specific example. The example chosen for analysis comprises a tunnel with UV sources located on the walls; an object, in this case a slab pre-sprayed with H$_2$O$_2$, is imagined to move through the tunnel at constant velocity. Predictions are obtained and presented here of the disinfection of the surfaces of the object as it moves through the tunnel.

6.2 Model description

6.2.1 General equations

It was seen in Chapter 3 that the disinfection kinetics of *B. subtilis* spores exposed to UV could be represented by first order kinetics:

$$\left( \frac{N}{N_0} \right) = e^{-kF}$$

(3.1a)
This was also the case for UV + 1% H₂O₂:

\[
\left( \frac{N}{N_0} \right) = e^{-k_1F}
\]  

(3.1b)

Equations (3.1a) and (3.1b) are in one sense limiting cases. To predict the disinfection performance in which a fraction of the surface, \( f_c \), was covered by 1% H₂O₂ spray, equations (3.1a) and (3.1b) can be combined to produce:

\[
\left( \frac{N}{N_0} \right) = f_c \cdot e^{-k_1F} + (1 - f_c) \cdot e^{-kF}
\]  

(6.1)

A zonal shielding model was developed in Chapter 4; this allowed equation (3.1) to be applied to surfaces which shielded micro-organisms to incident UV. Denoting the kinetic disinfection function in generalised terms as \( g \), i.e. :

\[
\left( \frac{N}{N_0} \right) = g(F)
\]

the disinfection performance can be predicted for a surface represented by \( n_z \) zones in which the fraction of the total number of micro-organisms on the surface, \( f_j \), and the surface view factor, \( \alpha_j \), are known in each zone:

\[
\left( \frac{N}{N_0} \right) = \sum_{j=1}^{n_z} f_j \cdot g(F_s \cdot \alpha_j)
\]  

(4.8)

where

\[
F = F_s \alpha_j
\]

\[
F_s = \text{UV fluence incident on surface}
\]
The UV intensity model developed in Chapter 5 allowed the UV intensity incident on a surface, $I_s$, to be calculated. For a given exposure time, $t$, the UV fluence received on the surface of the object is

$$F_s = I_s \cdot t$$

Once the fluence received by the surface is known then equation (4.8) can be applied.

In most real applications, the UV intensity will vary over the surface of an object. In such situations, in order to apply equation (4.8) it will be necessary to know the UV intensity distribution on the object surface. Methods of achieving this are described in Chapter 5 in which UV intensity field models were solved numerically. It was seen that in order to apply the model, the object surface must be divided into areas in which the UV intensity is considered to be uniform. The UV intensity is calculated at the centres of these areas, e.g.

![Figure 6.1 Surface area zones of constant UV intensity](image)

Once $I_s$ was calculated for each of these surface areas by the UV intensity field model, the survival after a specified exposure time, $t$, in each of these surface areas was calculated by equation (4.8):

$$\frac{N}{N_0} = \sum_{j=1}^{n_s} f_j \cdot g(I_s \cdot t \alpha_j)$$

(4.8')

If these surface areas are equal then the survival for the whole surface, comprised of $n_w$ surface areas, is calculated by the following equation:
For an object comprising $n_{so}$ surfaces, the survival on the whole object is given by:

$$y_{obj} = \sum_{k=1}^{n_{so}} a_k \cdot y_{surf_k}$$  \hspace{1cm} (6.3)

where

$$a_k = \frac{\text{surface area of surface } k}{\text{surface area of object}}$$

6.2.2 Example

The system of equations thus described are versatile enough to enable them to be applied to any geometrically defined object present in any UV field. To demonstrate the application of the equations a specific example is considered below.

Previously in Chapter 5 only stationary objects in stationary UV fields were considered. In this example an object having the geometry of a slab is assumed to travel through a spray disinfection field which is an open-ended 'tunnel' comprised of two walls either side of the object. The object travels at constant velocity through this tunnel. The tunnel is 10 metres in length and each wall is made up from 10 panel sections, each 1 metre in length, which have an identical arrangement of UV lamps, as shown in Figure 6.2.

The model micro-organism present on the surface of the slab is taken to be $B. subtilis$ spores, allowing the application of equations (3.1a) and (3.1b) using the parameters
FIGURE 6.2 Disinfection tunnel arrangement
determined from the experiments described in Chapter 3; \( k = 2.13 \times 10^{-2} \text{ (Jm}^{-2})^{-1}; \ k_1 = 0.129 \text{ (Jm}^{-2})^{-1} \).

The slab-shaped object is assumed to travel down the centre of the tunnel. The 6 surfaces of the slab have been numbered for reference. The slab has a height of 2 metres with a width and breadth of 0.25 metres each. A Cartesian system of co-ordinates has been applied to the disinfection tunnel with \( z = 0 \) being level with the bottom of the slab. The origin is located at the start of the tunnel on the left hand wall (see Fig. 6.2a). The slab-shaped object and the UV sources have been located so as to achieve a symmetrical UV intensity field on the object. This means that the opposite surfaces of the slab, i.e. sides 1 & 2, sides 3 & 4, and sides 5 & 6 have identical UV intensity patterns.

The UV sources used in the disinfection chamber are considered to be Voltarc G30 UV sources, as used in the experimental disinfection chamber described in Chapter 2 and used to generate the results in Chapter 3. These sources have an arc length of 0.35 m, a diameter of 1.5 cm and emit 13.1 W at 254 nm. The model had a provision for altering the arrangement of the UV sources on the wall panels. The first arrangement used is shown in Figure 6.3. This shows 2 UV sources positioned horizontally on the wall panel, each 0.25 m above and below the top and bottom of the slab, i.e. 2.50 m apart.

In the example considered below, the slab-shaped object will travel through the disinfection chamber for periods of time necessary to achieve certain levels of disinfection and the residence time in the disinfection chamber will affect the UV fluence received. In addition to investigating the effect of residence time on disinfection performance, the effect of changing the arrangement of UV lamps in the chamber by increasing the number of evenly-spaced UV lamps on each wall section will be investigated. Also, the effect of changing the orientation of the UV lamps from horizontal to vertical will be considered.
FIGURE 6.3 UV source arrangement on disinfection tunnel wall
In order to apply equation (6.3) it was necessary to know the UV fluence that each surface zone has received during its passage through the disinfection chamber. The UV intensities on the object surface will change as the object moves through the disinfection chamber. As the equations cannot be integrated it is necessary to solve the equations numerically. This is done by assuming that the object passes through sections of the disinfection tunnel in which the UV intensity is constant. This is shown in Figure 6.4. The greater the number of these sections the better the approximation. The UV intensity at specified points on the object surface is then calculated using the program

\[ F = \sum_{a=1}^{n_{sc}} F_a \]  \hspace{1cm} (6.4)

If the object is travelling at a constant velocity through sections of disinfection chamber which are of equal length then equation (6.4) can be rewritten as:

\[ \overline{F} = \sum_{a=1}^{n_{sc}} F_a \]  \hspace{1cm} (6.4)
where $t_s$ = residence time in a section of disinfection chamber where the UV intensity is constant

$I_a$ = UV intensity in tunnel section $a$

Equation (6.5) can be re-written as

$$\bar{F} = t_s \sum_{a=1}^{n_{sc}} I_a$$

(6.5)

$$\bar{F} = t_c \cdot \bar{I}$$

(6.6)

$$\bar{I} = \frac{\sum_{a=1}^{n_{sc}} I_a}{n_{sc}}$$

(6.7)

where

$\bar{I}$ = average UV intensity on the surface area during the passage through the disinfection chamber

$t_c$ = residence time of the object in the disinfection chamber

The computer program SLABRES (Appendix 1) uses the calculated fluence from equation (6.6) to determine the surviving fraction of micro-organisms in a surface area using equation (6.1). The program then determines the overall surviving fraction on each side of the slab and the whole surface of the slab using equations (6.2) and (6.3).
6.3 Results

From the arrangement of UV sources shown in Figure 6.3, the computer program SLABAV calculated the average UV intensity, \( \bar{I} \), over the surfaces of the slab-shaped object and the results are shown in Figure 6.5. The vertical surfaces facing the UV sources, sides 1 and 2, have a UV intensity approximately double the UV intensity on the other 4 surfaces of the slab, with the UV intensity decreasing towards the centre of the slab. The other surfaces of the slab have a more even UV intensity across their surfaces.

The Cartesian co-ordinates used in this figure are local co-ordinates to the slab surfaces. The origin of these co-ordinates is located in the top left hand corner of each surface. The greatest variation of UV intensity occurs on sides 1 and 2. By varying the residence time spent in the disinfection tunnel, \( t_e \), the program SLABRES predicted the disinfection levels on each of the 6 surfaces of the object and the object as a whole. These results are shown in Figure 6.6 which shows the residence times necessary to achieve a given disinfection performance. As the UV intensity field is identical on opposite surfaces of the slab, the disinfection level for these opposite surfaces will be identical.

The results shown in Figure 6.6 are for an object whose surfaces provide no shielding to incident UV. It is possible to use the surface shielding parameters estimated for various surfaces in Chapter 4 to predict the disinfection performance that would be achieved if the surfaces of the slab-shaped object exhibited various degrees of shielding against incident UV. Figure 6.7 shows the disinfection level that would be produced for the object if its surfaces provided the same degree of shielding to UV as beef steaks (from the results of Stermer et al., 1987) and dry Grades 2 and 6 filter papers (Whatman). The disinfection performance for surfaces providing no shielding is also shown for comparison.
FIGURE 6.5 UV intensity fields on surfaces of the slab in the disinfection tunnel
FIGURE 6.6  Disinfection level produced on slab-shaped object in disinfection chamber employing horizontal UV source arrangement
FIGURE 6.7 Disinfection levels produced on slab-shaped object exposed to UV in the disinfection chamber for different surface exposure factors
In order to see the effect of only partial coverage of a surface with 1% H$_2$O$_2$ spray on disinfection performance, the degree of coverage by spray was varied between 0 and 100%. It was assumed that the object provided no shielding to micro-organisms on the surface. The results are shown in Figure 6.8 and show that as coverage is increased from 0 to 100%, the residence time in the tunnel necessary to achieve identical levels of disinfection decreases to approximately 0.17 times the previous value.

As previously mentioned, the arrangement of the UV sources on the walls of the disinfection tunnel could be varied. Many variations could have been accommodated by the model but in the example described below the effect of increasing the number of horizontal UV sources on the walls was considered. The arrangement used, shown in Figure 6.9, was similar to that shown in Figure 6.3 except that the top and bottom UV sources were located 2 metres above and below the top and bottom surfaces of the slab respectively. Additional UV sources could be evenly distributed between the top and bottom UV sources. The number of UV sources per tunnel section of constant UV intensity was varied between 4 (i.e. 2 per wall) and 20 for a constant residence time in the disinfection chamber of 1000 seconds. The results are shown in Figure 6.10 where it is seen that increasing the number of sources above 8 makes no difference to the disinfection performance for the whole slab.

A further variation in the arrangement of the UV sources was considered. The orientation of the UV sources was changed so that the UV sources were vertically positioned on the walls of the disinfection chamber. Two UV sources per wall per unit tunnel section were employed with the centres of the UV lamps level with the top and bottom of the slab-shaped object in the centre of each 1m wall panel section. Using this arrangement, the average UV intensity for each surface area was calculated using SLABAV and the disinfection levels produced calculated using SLABRES. The UV intensity fields produced on the object's surfaces are shown in Figure 6.11. The disinfection level produced on the slab-shaped object when there is no shielding to incident UV is shown in Figure 6.12.
FIGURE 6.8 Disinfection level produced on slab-shaped object in the disinfection chamber when exposed to UV and 1.0 w/v% H₂O₂ spray of differing fractional surface coverage.
6.4 Discussion

Two arrangements of UV source orientation were considered - horizontal and vertical. A comparison of Figures 6.6 and 6.12 shows that the horizontal UV source arrangement produced the greater overall disinfection performance for the same residence time. The disinfection rates on the individual surfaces using the horizontal arrangement showed less variation than was the case with the vertical UV source arrangement. Figure 6.12 shows that the disinfection rates on the vertical surfaces of the object (sides 1 - 4) were faster using the vertical source arrangement than the corresponding disinfection rates for the horizontal arrangement. However as the UV intensity on the top and bottom surfaces (sides 5 and 6) produced was far lower, the disinfection rate for the whole object tends to that of sides 5 and 6 as the survival level
FIGURE 6.10 Disinfection level achieved in disinfection tunnel using different numbers of UV lamps per repeatable tunnel section
FIGURE 6.11  UV intensity on slab-shaped object in disinfection chamber using vertical UV source arrangement
FIGURE 6.12 Disinfection rate on slab using vertical UV source arrangement in disinfection tunnel
on the other 4 sides of the slab tends to zero. This result demonstrates that it is important to choose an arrangement of UV sources leading to approximately even UV intensities on the object surfaces. This avoids the situations occurring where overall disinfection rate is limited by individual surfaces.

These results show that the UV sources are best employed in a vertical arrangement for the vertical surfaces of the slab (sides 1 - 4) and best employed in a horizontal arrangement for the horizontal surfaces of the slab (sides 5 - 6). Therefore a prudent design would include both horizontal and vertical UV sources to ensure a more even UV intensity over the surfaces of the object.

Figures 6.6 and 6.12 show the disinfection level produced by a specified residence time within the disinfection chamber. The disinfection level shown is based on a calculated UV fluence. Thus the effect of doubling the UV intensity is to produce the same disinfection levels at half the residence time. Therefore the disinfection curves shown can be used to predict the disinfection levels that would be achieved for different UV intensities using the same UV source arrangement. However if the number of UV sources or their locations are changed then the UV intensity fields must be recalculated.

Figure 6.10 shows an example of where increasing the number of UV sources does not produce a great improvement in the disinfection level. In the case considered, increasing the number of UV sources had relatively little effect on the top and bottom surfaces of the slab. Thus the disinfection levels on the vertical surfaces become much greater but the overall disinfection rate for the object becomes limited by the top and bottom surfaces whose disinfection rates are not increasing with additional UV sources. One possibility not considered here was to situate UV sources on the floors and ceiling of the tunnel.
Figure 6.7 showed the effect of surface shielding from incident UV on the disinfection level obtained. It is seen that in the cases considered, the disinfection level obtained was greatly reduced where surface shielding occurred. It is also seen from Figure 6.7 that the disinfection rate becomes smaller at higher fluences and tends to 'tail off' to a plateau region. In these cases increasing the UV intensity on the object, either by increasing the number of UV sources or the residence time, will produce little to no additional disinfection of the object. One example of this is the surface of beef steaks (from the results of Sterner et al., 1987). The zonal shielding model fitted a third zone in which the micro-organisms were virtually completely shielded from incident UV. Therefore the number of viable micro-organisms in this zone will be fairly insensitive to the UV dose applied. This again shows that simply increasing the number of UV sources will not necessarily increase the disinfection performance. A slight increase in disinfection performance may not be economically worthwhile when balanced against the capital and operating costs of additional UV sources. Thus a designer should chose the UV source arrangement carefully.

Figure 6.8 shows that the fraction of the object’s surfaces covered by 1% H$_2$O$_2$ spray when in the presence of UV greatly influences the disinfection level obtained. It was seen in Chapter 3 that the disinfection rate for _B.subtilis_ spores exposed to UV + 1% H$_2$O$_2$ was greater than that when the spores were exposed to UV alone. If a surface is not fully covered with H$_2$O$_2$ spray then the covered surface areas are disinfected more quickly and the microbial count tends to zero in those areas. The remaining uncovered areas are disinfected less quickly and as the microbial count in the spray-covered surface areas tends to zero, the disinfection rate tends to that in the uncovered surface areas. This is clearly seen in Figure 6.8 for surfaces with uncovered areas. The gradient of the disinfection curve for these surfaces tends to that of the disinfection curve for UV alone as the residence time increases.

This result shows that it is very important to obtain full coverage of the object’s surfaces with H$_2$O$_2$ spray because the disinfection performance is very sensitive to the
fractional coverage of the surface, e.g. note the difference between 99% and 100% coverage of the surface on the disinfection level obtained. The sensitivity of the disinfection performance to the fractional coverage of the surface can also explain why the experimental disinfection results obtained with peroxide sprays within the disinfection chamber (see Chapter 3) were more variable than those obtained with UV as the sole disinfectant.

6.5 Conclusions

It is seen from this example that it is possible to predict the disinfection rate for a specific micro-organism on an object if the incident UV intensity and surface shielding are known. All the predictions made here assumed that the surfaces were contaminated with *B. subtilis* spores which Toledo *et al.* (1973) claimed was one of the most resistant micro-organisms to H₂O₂. Real surfaces are therefore likely to be contaminated with more susceptible organisms and therefore the predictions here are conservative.

This approach is quite intensive and would only be used as a more detailed design stage after initial calculations using simpler line source with spherical emission (LSSE) UV intensity models which would not take into account such factors as the view factors and surface shielding factors on an object surface when calculating the disinfection level. These would be sufficient to provide very approximate values of disinfection levels.

The example considered here demonstrated that it is important to avoid large differences in the disinfection rates on the object as the disinfection rate tends to the lowest disinfection rate as the overall survival is reduced. This can be ensured with careful arrangement of the UV sources. The UV intensity field models can be applied to any object geometry and provide a useful tool in ensuring an even UV intensity field.
pattern across the surfaces of the object. When using the combined UV + 1% H₂O₂ spray disinfection system, the results obtained showed that it was important to achieve full coverage of the object surface in order to avoid variable disinfection rates. This must be balanced against over use of peroxide spray due to legal limits on the levels of hydrogen peroxide that are permitted in foodstuffs for example, which is 0.1 ppm (Toledo et al., 1973).

6.6 Notation

\[ a_k = \text{fraction of total surface area of object on surface } k \]
\[ f_c = \text{fraction of surface covered by spray} \]
\[ f_j = \text{fraction of micro-organisms in zone } j \text{ (zonal shielding model)} \]
\[ F_s = \text{fluence incident at surface} \]
\[ g(F) = \text{function giving fractional survival in terms of fluence received by micro-organisms} \]
\[ I_s = \text{UV intensity incident on surface} \]
\[ k = \text{multi-target equation rate coefficient} \]
\[ k_1 = \text{first-order rate coefficient} \]
\[ N = \text{microbial surface concentration} \]
\[ N_0 = \text{initial microbial surface concentration} \]
\[ n = \text{number of targets in multi-target equation} \]
\[ n_{sc} = \text{number of sections of constant UV intensity comprising the disinfection tunnel} \]
\[ n_{so} = \text{number of surfaces on an object} \]
\[ n_{sp} = \text{number of surface areas of constant UV intensity comprising a surface} \]
\[ n_z = \text{number of zones (zonal shielding model)} \]
\[ t = \text{exposure time} \]
\[ t_e = \text{residence time in disinfection tunnel} \]
\( y \) = fractional survival

\( \bar{y} \) = overall fractional survival

\( \bar{y}_{obj} \) = overall fractional survival on an object

\( \bar{y}_{surf} \) = overall fractional survival on a surface

\( \alpha_j \) = surface view factor in zone \( j \) (zonal shielding model)
7. OVERALL CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

7.1 Overall Conclusions

1. The experimental techniques developed in this work enabled the inactivation of *B. subtilis* spores associated with solid surfaces to be investigated. Spore inactivation was brought about by UV irradiation, peroxidation and ozonation applied either singly or in combination. No single method of spore deposition was universally applicable either for all surfaces or for all the disinfection treatments examined.

2. Kinetic models, either first order or multi-target, were fitted to the experimental data and enabled comparisons of the disinfection treatments to be made. Combinations of UV and H₂O₂ were shown to act synergistically in inactivating spores and the greatest synergy was displayed at a H₂O₂ concentration of 1.0% w/v.

3. Ozone exhibited sporicidal activity against surface-associated *B. subtilis* spores when in gaseous form but not as a saturated aqueous solution. This was thought to be due to an interaction between the surface and aqueous ozone.

4. UV was shown to be more effective against *B. subtilis* spores on surfaces such as membranes than on others such as fibrous filter papers. This was attributed to the micro-topography of the surface affording the spores a measure of protection from incident UV.

5. A zonal shielding model was developed in which surface associated micro-organisms were assumed to be distributed between a finite number of zones each having a characteristic Exposure Factor which defined the degree of protection from incident UV afforded by that zone. Non-linear programming techniques were employed to
obtain solutions of the microbial distribution and of the Exposure Factors for each zone. In practice it was found that in all cases three zones were sufficient to describe the experimental data.

6. A mathematical UV field model was developed to predict the surface intensities on objects of regular geometry located within a UV field. Bioassay results and radiometer readings showed good agreement with the model.

7. The combination of the UV field model with experimentally obtained disinfection kinetics allowed predictions to be made of the rate of inactivation of \textit{B. subtilis} spores on the surfaces of objects known geometry treated with either UV or UV and H\textsubscript{2}O\textsubscript{2}. The model developed was sufficiently versatile to enable application to any object of regular geometry in UV fields generated by various combinations of UV sources.

7.2 Suggestions for further work

7.2.1 Deposition of Test Micro-organisms on Surfaces

Whilst enabling useful kinetic data to be obtained for the inactivation of \textit{B. subtilis} spores on a variety of surfaces, the experimental techniques employed here did reveal some limitations. Loss of spores occurred from surfaces due to physical wash-off by aqueous hydrogen peroxide but also occurred under conditions when wash-off could not have been the cause. The actual cause was never determined but was related to the use of the air-atomising nozzle and might possibly have involved electrostatic forces.

A method of immobilising a test organism in a suitable matrix could eliminate these difficulties. One possible contender in this regard is pectin. The test organism would need to be suspended in pectin whilst the latter was in molten form. The pectin would then be sprayed onto solid objects whereupon it would harden to produce a gel, in a manner similar to that employed in the food industry to glaze confectionery. Following
irradiation, the gel film would be peeled off and the organism recovered by treating with the enzyme pectinase. Preliminary studies have shown that \textit{B. subtilis} spore viability was not adversely affected by pectinase. In addition to reducing, or even eliminating, physical loss of the test organism, the possibility also exists that the matrices produced would be similar to the biofilms found in natural environments in which micro-organisms are present both on the surface and throughout the depth of the film (Blenkinsopp and Costerton, 1991). Moreover, the UV absorptive properties of the films could be modified by, for example, incorporating inert solid material such as talc. Another possibility is the prospect of employing species other than \textit{B. subtilis} spores as test organisms. The use of micro-organisms more representative of the microflora of natural surfaces would enable more accurate estimates to be made of the conditions necessary to achieve required levels of disinfection. Spores are in general more resistant than vegetative forms (Block, 1983). Preliminary results obtained during the course of this work showed that a number of common surface-associated microorganisms were unable to survive the desiccation which occurred during deposition of the organisms onto the test surfaces, but entrapment of such organisms within an aqueous gel might well significantly improve their survival.

7.2.2 Verification of the Zonal Shielding Model

The application of the zonal shielding model to materials providing a relatively high degree of shielding yielded solutions which implied the existence of discrete zones characterised by Exposure Factors which approached zero. Whilst it was acknowledged in Chapter 4 that surfaces would in reality comprise a distribution of exposure factors, the model nonetheless, predicates the existence of a fraction of the microbial population which would remain viable even at very high UV fluences. This prediction would need to be experimentally verified using materials which offered different degrees of surface shielding and would require the exposure of these materials to UV fluences of increasing magnitude whilst determining microbial survival.
7.2.3 Electrostatic Spraying

The work carried out here to investigate the effect of charging sprays of disinfectants on the disinfection levels obtained on surfaces was encouraging but inconclusive. This was primarily due to the shortcomings in the design of the disinfection chamber which promoted charge leakage and which resulted in a highly turbulent air flow pattern. Whilst use of the chamber provided necessary containment of the oxidising sprays, a fundamental redesign of the chamber would need to be undertaken to enable consistent results to be obtained with charged sprays. In particular, both the nozzle and the object would need to be more remote from surrounding structures which might provide a conduit to earth.

7.2.4 Predicting impact rates from spray nozzles

In order to predict the impact rate of sprays from a nozzle on a target surface, it would be necessary to have a model which could predict the spray density, drop size distribution and velocities at any given spatial location. If an air-atomised nozzle was used then the effect of the surrounding air on the spray droplets would increase with decreasing drop size until they are dominated by the surrounding air, as was the case with atomised sprays (Boothroyd, 1971). Further complications would arise when the air flow from the nozzle became turbulent at a given distance from the nozzle. To predict the impact on an object, it would be necessary to know the air-flow pattern around the object.

Commercially available computational fluid dynamic (CFD) packages could be used to solve the time-averaged Navier-Stokes equations describing the coupled air-spray droplet system. Certain parameters would need to be experimentally determined for the model. These include the drop size distribution of the spray at known distances from the nozzle, the droplet density across the spray angle and the velocity distribution of the drops. The model would allow the effects of variations in key design parameters
such as distance between nozzle and target, feed air pressure to the nozzle, electrostatic-charging of the nozzle etc. on disinfection performance to be investigated.
A1.1 Zonal Shielding model

A1.1.1 Background

The program ZONESHLD attempts to find a Karesh-Kuhn-Tucker point which solves the nonlinear programming problem described in Chapter 4. This listing of ZONESHLD uses first-order kinetics to describe unshielded UV disinfection kinetics for *B.subtilis* spores. However it is possible to employ other kinetic expressions. These should be placed in the OBJFUN subroutine (at the end of the program).

The number of variables to be solved for will depend on the number of active inequality constraints. The matrix, $X$, of the variables to be solved for consists of the values of $f$ and $\alpha$ in each of the $n_z$ zones, $\lambda$, the Lagrangian multiplier for the equality constraint (4.9) and the Lagrangian multipliers, $\mu$, for the active inequality constraints (4.10).

$$X = \begin{bmatrix} f_1 \\ \vdots \\ f_{n_z} \\ \alpha_1 \\ \alpha_{n_z} \\ \lambda \\ \mu_1 \\ \mu_{2n_z} \end{bmatrix}$$

Thus $X$ can contain between $(2n_z + 1)$ and $(4n_z + 1)$ variables to be solved for.

The form of equation (4.11) which describes the sum of the square of the errors, $S$, does not readily lend itself to differentiation. Approximate values of the derivatives were obtained using centre-spaced finite difference methods, which are derived from the Taylor series (Bajpai *et al.*, 1988). The first and second derivatives of the Lagrangian, $L$, with respect to two variables, $x_1$ and $x_2$, are given by the following equations:
\[
\left( \frac{\partial V}{\partial x_i} \right) \approx \frac{L(x_i + h) - L(x_i - h)}{2h} \quad (A1.1)
\]
\[
\left( \frac{\partial^2 L}{\partial x_i^2} \right) \approx \frac{L(x_i + h) - 2L(x_i) + L(x_i - h)}{h^2} \quad (A1.2)
\]
\[
\left( \frac{\partial^2 L}{\partial x_i \partial x_j} \right) \approx \frac{L\left( \frac{x_i + h}{x_2 + h} \right) - L\left( \frac{x_i - h}{x_2 + h} \right) + L\left( \frac{x_i - h}{x_2 - h} \right) - L\left( \frac{x_i + h}{x_2 - h} \right)}{4h^2} \quad (A1.3)
\]

where \( h \) is the finite difference mesh spacing. Consistent results were obtained with values of \( h \) between \( 10^{-3} \) and \( 10^{-5} \).

The nonlinear simultaneous equations, \( \nabla L = 0 \), must be solved by iterative methods. Using Newton's method (Bajpai et al., 1988), the following equations can be written:

\[
\nabla^2 L(X_k) p_k = -\nabla L(X_k) \quad (A1.4)
\]

\[
X_{k+1} = X_k + p_k \quad (A1.5)
\]

where \( p_k \) is the step to be taken from the \( k \)th approximation to \( X \).

The initial values of \( X \) are set by random numbers in the INIT subroutine. At each approximation to \( X \), \( \nabla^2 L(X_k) \) and \( \nabla L(X_k) \) can be determined using equations (A1.1) - (A1.3). Equation (A1.4) is then solved for \( p_k \) using Gaussian elimination (Bajpai et al., 1987) and the next approximation to \( X \) is then calculated using equation (A1.5). Successive approximations to \( X \) are determined by this method until the root geometric mean of the residuals of \( \nabla L = 0 \) is less than or equal to a specified tolerance, CONVTOL.

If the program is successful then the final value of \( X \) is displayed, together with the value of \( S \) and the root geometric mean of the residuals. During processing, if the program cannot proceed further due to encountering a singular matrix or an infeasible point (e.g. a point which required the log of a negative number to be calculated) then the program will terminate and display the point at which lowest value of the sum of the square of the errors, \( S \), was encountered.

ZONESHLD was written in a compilable BASIC language called POWERBASIC.
A1.1.2 Program listing

REM
REM ZONESHLD
REM
REM A program to solve the Zonal Shielding model by searching
REM Karesh-Kuhn-Tucker points.
REM
SDYNAIC
DEFDBL A-Z 'default to double precision
REM
F = 0.000 'objective function (Lagrangian)
S = 0.000 'sum of the square of the errors
REM
NAC% = 0 'number of active constraints
NZ% = 4 'number of zones
N% = 2*NZ%+1+NAC% 'number of variables on which objective function
' 'depends
REM
NMAX% = 4*NZ%+1 'maximum number of variables that may be solved for
REM
Active constraints
REM
DIM AC%(2*NZ%)
DIM G(4*NZ%)
REM
Experimental data (shielded surface)
REM
NP% = 26 'number of data points
DIM FS(NP%)'surface fluence at each data point
DIM YEXP(NP%)'log10 survival at each data point
REM
K1 = 2.13E-2 '1st order constant for unshielded kinetics
KL = 0.434294481 'log base conversion factor
REM
DIM X(NMAX%) 'variables (maximum number possible)
DIM DF(NMAX%) '1st derivatives
DIM D2F(NMAX%,NMAX%) '2nd derivatives
REM
ONE = 0.000 'objective function at each of the 4 points
ONW = 0.000 'used to calculate the second derivatives
OSE = 0.000
OSW = 0.000
REM
FE = 0.000 'objective function at the 2 points used for 1st
FW = 0.000 'derivative
REM
DIM XE(NMAX%) 'working arrays
DIM XW(NMAX%)
REM
DIM XNE(NMAX%) 'working arrays
DIM XNW(NMAX%)
DIM XSE(NMAX%)
DIM XSW(NMAX%)
REM
*********** finite difference mesh spacing ***********
H = 1E-5
*********** end finite difference mesh spacing ***********
REM
DIM P(NMAX%) 'step direction from Newton-Raphson method
REM
RGM = 0.0 'root geometric mean
CONVTOL = 2E-2  convergence tolerance
TOL = 1E-3  variable tolerance

REM DIM F1(NMAX%)
REM DIM XI(NMAX%)
REM DIM PR(NMAX%)
REM DIM R(NMAX%)
REM DIM A(NMAX%,NMAX%) matrices for Gaussian elimination
REM DIM B(NMAX%)
REM DIM A0(NMAX%,NMAX%)
REM DIM B0(NMAX%)
REM DIM XBEST(NMAX%)
REM RGMBEST = 1E6
REM SBEST = 1E6

REM Define error trapping code (declaratives)
ON ERROR GOTO 9000

REM ******************************************************
REM INITIATE VARIABLES
REM ******************************************************

REM Set up the experimental survival data for the shielded surface
CALL EXPER(FSQ,YEXP(),NP%)

REM Set up the initial point
CALL INIT(X(),N%)

REM ******************************************************
REM STEP 1
REM ******************************************************

REM Set the flags to 0 (0 = no, 1 = yes )
SOLFLAG% = 0 solution reached flag
ERRFLAG% = 0 error encountered flag

REM ITER2% = 1
DO WHILE SOLFLAG% = 0
N% = 2*NZ%+1+NAC% number of variables to solve for

CLS
LOCATE 1,1
PRINT "Pass number ";ITER2%; " through the main loop"
PRINT PRINT NAC%; " active inequality constraints"
PRINT INPUT Z9
IF Z9 <> 0 THEN STOP

REM ITER% = 1 counter for iterations performed in step 2 loop
REM
REM Find df(X) at the point at the start of the step 2 loop
REM GOSUB 1000 calculate 1st order differentials
REM CALL CONVRES1J(DF0,N%,RGM)
REM Main loop - perform this loop until the residuals of df(X)=0 have
REM been reduced to within the specified tolerance
REM
REM ***********************************************
REM STEP 2
REM ***********************************************
REM
DO WHILE RGM > CONVTOL
REM
CLS
LOCATE 1,1
PRINT "Iteration:"; ITER%" in step 2 loop"
LOCATE 3,1
PRINT "RGM = ",RGM
LOCATE 5,1
PRINT "Current X matrix is"
PRINT
FOR K%=1 TO N%
PRINT K%,X(K%)
NEXT K%
END IF
PRINT
PRINT "Hit enter to continue"
INPUT ZI
IF ZI <> 0 THEN STOP
REM Not reached the solution. Thus must perform another iteration.
REM
IF S < SBEST THEN
FEAS%=1
FOR 1%=1 TO 2*NZ%
IF X(1%) < -TOL OR X(1%) > ( 1.000 + TOL ) THEN
FEAS%=0
END IF
NEXT 1%
REM
IF FEAS%=1 THEN 'no bounds violated
RGMBEST = RGM
SBEST = S
FOR J%=1 TO N%
XBEST(J%) = X(J%)
NEXT J%
END IF
END IF
REM
REM Calculate the 2nd derivatives of the equations using finite differences
REM
REM
GOSUB 2000 'calc 2nd order derivatives
REM
REM
REM Solve for the step direction using Gaussian elimination
REM
REM
REM First set up -df(X) in F1 array
REM
REM
FOR K%=1 TO N%
F1(K%) = -DF(K%)
NEXT K%
REM
REM Now call the Gaussian elimination routine to solve for P
REM
REM
GOSUB 5000 'Gaussian elimination routine
REM
REM Determine how far to move in the search direction and then perform
REM the move
REM
CALL STEPSIZE(X(),P(),N%)

REM
REM Calculate dt(X)
REM
GOSUB 1000  'calc 1st order derivatives
CALL CONVRESD(DF(),N%5,RGM)  'determine RGM
REM
ITER% = ITER% + 1
REM
REM End of stage 2
REM
LOOP
REM

*************************************************************
REM
REM STAGE 3
REM
*************************************************************
REM
REM Step 3 of the iterative set strategy
REM
REM Set up flags ( 0 = no, 1 = yes )
REM
300  GFLAG% = 0  'inequality constraints violated
      MUFLAG% = 0  'negative multipliers for inequality constraints
REM
REM Check to see if any inequality constraints have been violated
REM
FOR I% = 1 TO NZ%
REM
REM Check lower bounds
REM
IF X(I%) < -TOL OR X(I%+NZ%) < -TOL THEN
   GFLAG% = 1
END IF
REM
REM Check upper bounds
REM
IF X(I%) > (1.0000 + TOL) OR X(I%+NZ%) > (1.0000 + TOL) THEN
   GFLAG% = 1
END IF
REM
NEXT I%
REM
REM Check to see if there are any negative Lagrangian multipliers for
REM active inequality constraints.
REM
IF NAC% > ZERO THEN
REM
FOR I% = 1 TO NAC%
   IF X(2*NZ%+1+I%) < -TOL THEN
      MUFLAG% = 1
   END IF
NEXT I%
REM
END IF
REM
REM Check to see if solution reached
REM
IF GFLAG% = 0 AND MUFLAG% = 0 THEN
   SOLFLAG% = 1
   GOTO 500  'display final results and stop
END IF
REM
*************************************************************
REM
STEP 4A
REM
*************************************************************
Step 4a of the iterative set strategy

( Remove the inequality constraint with the largest negative multiplier)

\[
\text{LN}M\% = 0 \quad \text{number of the largest negative multiplier}
\]

\[
\text{VL}NM = 0 \quad \text{value of the largest negative multiplier}
\]

IF NAC\% > ZERO AND MUFLAG\% = 1 THEN

Find largest negative Lagrangian multiplier

FOR I\%=1 TO NAC\%

IF X(2*NZ%+1+I\%) < VLNM THEN

VLNM = X(2*NZ%+1+I\%)

LN\% = I\%

END IF

NEXT I\%

Remove this multiplier from the active set

IF LN\% < 2*NZ\% THEN

Shift constraints up 1 in the matrix

FOR I\%=LN\% TO NAC\%+1

K\% = 2*NZ\%+1

X(K\%+I\%) = X(K\%+I\%+1)

AC\%(I\%) = AC\%(I\%+1)

NEXT I\%

X(4*NZ%+1) = 0.000000

AC\%(2*NZ\%) = 0.000000

NAC\% = NAC\% - 1

END IF

Step 4b of the iterative set strategy

( Add all violated inequalities to the active set )

Check lower bounds

FOR I\%=1 TO 2*NZ\%

IF X(I\%) < -TOL THEN

add to active set

NAC\% = NAC\% + 1

AC\%(NAC\%) = I\%

set the variable to the bound value and its Lagrangian multiplier to 1

X(I\%) = 0.0000

X(2*NZ%+1+I\%+NAC\%) = 1.0000

END IF

NEXT I\%

Check upper bounds

FOR I\%=1 TO 2*NZ\%

IF X(I\%) > (1.000 + TOL) THEN
REM add to active set
REM NAC% = NAC% + 1
AC%(NAC%) = 1 + 2*NNZ%
REM set the variable to the bound value and its Lagrangian multiplier to 1
REM IF
REM X(1%) = 1.00000
REM X(2*NNZ% + 1 + NAC%) = 1.00000
END IF
NEXT 1%
REM
REM Reset initial estimates of all active multipliers to 1
REM
REM X(2*NNZ% + 1) = 1.00000
REM FOR 1% = 1 TO 2*NNZ%
REM X(1% + 2*NNZ% + 1) = 0.0000
NEXT 1%
REM
REM FOR 1% = 1 TO NAC%
REM X(2*NNZ% + 1 + 1%) = 1.00000
NEXT 1%
REM
REM Return to Step 2
REM
ITER2% = ITER2% + 1
REM LOOP
REM STOP
REM
*****************************************************************************
REM SOLUTION REACHED - DISPLAY RESULTS
*****************************************************************************
REM
CLS
PRINT "Solution reached after ";ITER2%; " iterations"
PRINT "RGM; ";RGM; "; S = ";S
PRINT
REM FOR 1% = 1 TO 2*NNZ%
PRINT MAX(I%)%1
NEXT 1%
REM STOP
REM
*****************************************************************************
REM CALCULATE 1st ORDER DIFFERENTIALS
*****************************************************************************
REM
1000 H1 = 0.5 / H
REM
FOR 1% = 1 TO N%
REM FOR 1% = 1 TO N%
XE(1%) = X(1%)%1
XW(1%) = X(1%)%1
NEXT 3%
REM
XE(1%) = XE(1%)%1 + H
XW(1%) = XW(1%)%1 + H
REM
CALL OBJFUN(FE, XE())
CALL OBJFUN(FW, XW())
REM
DF(I%) = (FE - FW) * H1

REM
RETURN

REM
******************************************************************************
REM CALCULATE 2nd ORDER DIFFERENTIALS
REM******************************************************************************
REM Calculate the diagonal first (d2F/dx12)
REM H2D = 1 / (H'2)
REM
FOR I% = 1 TO N%
  FOR J% = 1 TO N%
    XE(J%) = X(J%)
    XW(J%) = X(J%)
  NEXT J%
REM
XE(I%) = XE(I%) + H
XW(I%) = XW(I%) - H
REM
CALL OBJFUN(FE,XE(I%))
CALL OBJFUN(FW,XW(I%))
CALL OBJFUN(FX(I%))
REM
D2F(I%,I%) = (FE - 2*F + FW) * H2D
NEXT I%
REM
Now calculate the rest of the 2nd order differentials
REM
H2 = 0.25 / (H'2)
REM
FOR I% = 1 TO N%
  FOR J% = 1 TO N%
    IF I% <> J% THEN
      NEXT J%
    END IF
  NEXT J%
REM
FOR A% = 1 TO N%
  XNE(A%) = X(A%)
  XNW(A%) = X(A%)
  XSE(A%) = X(A%)
  XSW(A%) = X(A%)
  NEXT A%
REM
XNE(I%) = XNE(I%) + H
XNE(I%) = XNE(I%) + H
REM
XNW(I%) = XNW(I%) - H
XNW(I%) = XNW(I%) + H
REM
XSW(I%) = XSW(I%) - H
XSW(I%) = XSW(I%) - H
REM
XSE(I%) = XSE(I%) + H
XSE(I%) = XSE(I%) - H
REM
CALL OBJFUN(ONE,XNE(I%))
CALL OBJFUN(ONW,XNW(I%))
CALL OBJFUN(OSW,XSW(I%))
CALL OBJFUN(OSE,XSE(I%))
REM
D2F(I%,I%) = (ONE - ONW + OSW - OSE) * H2
REM
END IF
NEXT J%
REM
RETURN
SEARCH for the step direction

A general program to perform gaussian elimination with pivoting on the system \( Ax = B \).

First set up the A and B matrices from the values specified.

Save the original A and B matrices - need these for residuals calculation.

5000 FOR I% = 1 TO N%
FOR J% = 1 TO N%
   \( A(I%, J%) = D2F(I%, J%) \)
   \( A0(I%, J%) = A(I%, J%) \)
NEXT J%

\( B(I%) = F1(I%) \)
\( B0(I%) = B(I%) \)
NEXT I%

Enter main loop

FOR \( I% = 1 \) TO \( N% - 1 \)

CALL PIVOT(\( A() \), \( B() \), \( P() \), \( N% \), \( I% \))

Now have the rows in the correct order after pivoting. Now perform Gaussian elimination to eliminate another variable:

CALL GAUSS(\( A() \), \( B() \), \( P() \), \( N% \), \( I% \))

NEXT I%

Finished the Gaussian elimination - calculate the results

\( P(N%) = B(N%) / A(N%, N%) \)

FOR I% = N% - 1 TO 1 STEP -1

\( T1 = 0.00 \) working variable
FOR \( J% = 1 \) TO \( N% \)
   \( T1 = T1 + A(I%, J%) * P(J%) \)
NEXT J%

\( P(I%) = (B(I%) - T1) / A(I%, I%) \)

NEXT I%

Restore A and B matrices for residuals calc

FOR I% = 1 TO N%
FOR J% = 1 TO N%
   \( A(I%, J%) = A0(I%, J%) \)
NEXT J%

\( B(I%) = B0(I%) \)

NEXT I%

Calculate the residuals

CALL RESID(\( A() \), \( B() \), \( P() \), \( R() \), \( N% \))

Enter main loop
FOR L% = 1 TO (N% - 1)
REM Perform the pivoting
REM CALL PIVOT(AQ,R,Q,PR,N%,L%)
REM Now have the rows in the correct order after pivoting. Now perform
REM Gaussian elimination to eliminate another variable.
REM CALL GAUSS(AQ,R,Q,PR,N%,L%)
REM NEXT L%
REM Finished the Gaussian elimination - calculate the results and exit
REM PR(N%) = R(N%) / A(N%,N%)
REM FOR I% = N%-1 TO 1 STEP -1
T1 = 0.00 'working variable
FOR J%=1%+1 TO N%
   T1 = T1 + A(1%,J%) * PR(J%)
NEXT J%
REM PR(I%) = (R(I%) - T1) / A(I%,I%)
P(I%) = P(I%) * PR(I%)
NEXT I%
REM end of routine
REM RETURN

REM ************************************************************
REM ERROR HANDLING (declaratives)
REM ************************************************************
REM An error occurred - possibly division by zero, or trying to take the log
REM of a non-positive number. Program has to stop. Show the user the
REM point at which the lowest value of S, the sum of the square of the
REM errors, was found.
REM REM 9000
CLS
PRINT "Program terminated with error ";ERR
PRINT PRINT "The point at which the lowest sum of the square of the errors was encountered was":
PRINT FOR I%=1 TO 2*NZ%
   PRINT I%,XBEST(I%)
NEXT I%
REM PRINT "S = ";SBEST; " RGM = ";RGMBEST
STOP
REM SUB INIT(X(),N%)
REM Set up the initial random values of X
REM SHARE N%:NAC%
REM Randomize the number of seconds there have been since midnight.
REM This should give a different set of initial values each time
REM the program is run.
REM RANDOMIZE TIMER
REM Randomized - now select the initial point

189
```plaintext
REM
FLAG%=0
DO WHILE FLAG%=0
  T1 = 0.0000
  FOR I%=1 TO NZ%-1
    X(I%) = RND
    T1 = T1 + X(I%)
  NEXT I%
REM
X(NZ%) = 1.000 - T1
IF X(NZ%) >= 0.000 AND X(NZ%) <= 1.000 THEN
  FLAGE%=1
END IF
LOOP
REM
Set alpha values
REM
FOR I%=1 TO NZ%
  X(I%+NZ%) = RND
NEXT I%
REM
X(N%)- 1.000   "initial estimate of lambda"
REM
Set the Lagrangian multipliers for the inequality constraints
to zero.
REM
FOR I%=2*NZ%+2 TO 4*NZ%-1
  X(I%) = 0.0000
NEXT I%
REM
CLS
PRINT "initial values:"
PRINT
FOR I%=1 TO N%
  PRINT I%,X(I%)
NEXT I%
PRINT
INPUT Z9
REM
END SUB
REM
SUB EXPER(FS%,YEXP%,NP%)
REM
Set up the experimental data to determine the sum of squares of the
errors by
REM
RESTORE 9998
REM
FOR K%=1 TO NP%
  READ FS(K%), YEXP(K%)
  YEXP(K%) = LOG10(YEXP(K%))
NEXT K%
REM
9998
DATA 177.0,3090,354.0,1774
DATA 708.0,0537,1770,3.631E-4
REM
DATA 88.5,0.4446, 1770,0.1702
DATA 354.0,0.09419,708.0,0.2032,1060,3.069E-3
DATA 1420,1.30E-4, 1770,1.019E-4
DATA 523,0.01129,1310,1.114E-3
DATA 278.0,0.05129,1310,1.514E-3
DATA 60.8,0.578,243.0,0.1567,486.4,3.635E-2
DATA 729.0,0.01148,1220,2.40E-3
REM
DATA 58.1,0.557,1160.412
```

190
DATA 232.0, 10.86, 464.0, 0.04677, 697.0, 0.01148

REM END SUB
REM
REM SUB PIVOT(A(),B(),XO,N%,L%)
REM Pivot the rows around so that the row with the largest coefficient
REM for the variable about to be eliminated appears in the top row.
REM
REM Find the row with the largest coefficient in the column of the
REM A matrix for the variable that is to be eliminated.
REM
DIM AT(N%)
ROW% = 0

FOR I% = L% + 1 TO N%
  IF ABS(A(I%,L%)) > ABS(A(L%,L%)) THEN
    ROW% = I%
  END IF
NEXT I%

IF ROW% > 0 THEN
  REM Need to pivot
  REM Save the old top row in a temporary array and then swap the rows
  REM around.
  FOR J% = L% + 1 TO N%
    AT(J%) = A(J%,L%)
    A(J%,L%) = A(ROW%,J%)
    A(ROW%,J%) = AT(J%)
  NEXT J%

  BT = B(L%)
  B(L%) = B(ROW%)
  B(ROW%) = BT
END IF

REM END SUB
REM
REM SUB GAUSS(A(),B(),XO,N%,L%)
REM Have pivotted the A matrix if necessary. Now must perform the
REM Gaussian elimination step to eliminate the variable in the L% column.
REM
REM Want to eliminate this variable from the subsequent N% - L% rows
REM which contain it.
REM
FOR I% = L% + 1 TO N%
  ELIM = A(I%,L%) / A(L%,L%)
  FOR J% = L% TO N%
    A(I%,J%) = A(I%,J%) - ELIM * A(L%,J%)
  NEXT J%
  B(I%) = B(I%) - ELIM * B(L%)
  NEXT I%

REM END SUB
REM
REM SUB RESID(A(),B(),XO,R,N%)
REM Calculate the residuals from the solution of the Gaussian elimination
REM
FOR I%=-1 TO N%
REM Calculate A.X - B = R (where X is the solution vector)
REM
R(I%) = 0.0000
REM
FOR J%=-1 TO N%
R(I%) = R(I%) + A(I%,J%) * X(J%)
NEXT J%
REM
R(I%) = R(I%) - B(I%)
NEXT I%
END SUB

SUB STEPSIZE(X(),p(),N%)
REM Must determine a suitable step size in the search direction. Choose
REM one which minimises the objective function
REM
BETA = 1.000
REM
Chosen the step size - now perform the step
REM
FOR I%=-1 TO N%
X(I%) = X(I%) + BETA * P(I%)
NEXT I%
END SUB

SUB CONVRESID(DF(),N%,RGM)
REM Calculate the root geometric mean
REM
RGM = 0.000
REM
FOR I%=-1 TO N%
RGM = RGM + DF(I%)^2
NEXT I%
REM
RGM = SQR(RGM)
END SUB

SUB OBJFUN(F(X))
REM Calculate the objective function at X
REM
SHARED YEXP(),FS(),N%,NZ%,K1,KL,NP%
SHARED NAC%,G(Q,AC%),S
REM
F = 0.00000
REM
FOR K%=1 TO NP%
REM Calculate Y (N/No) at the kth data point
REM
T1 = 0.0000  ' working variable
REM
FOR J%=-1 TO NZ%
T1 = T1 + X(J%) * EXP(-K1 * X(J%+NZ%) * FS(K%))
NEXT J%
IF $T_1 > 0$ THEN
  $T_1 = \log_{10}(T_1)$
ELSE
  $T_1 = -10.000$
END IF

REM $Y_K = T_1$
REM
$F = F + (Y_K - Y_{\exp(K)})^2$
REM
NEXT $K$

REM $S = F$
REM
REM Add the equality constraint's contribution to the Lagrangian
REM $T_1 = 0.000$
REM
FOR $I% = 1$ TO $N_Z$
  $T_1 = T_1 + X(I%)$
NEXT I%
REM
$F = F + X(2\cdot N_Z + 1) \cdot T_1$
REM
REM Add the contribution from the inequality constraints
REM
FOR $I% = 1$ TO $2\cdot N_Z$
  $G(I%) = -X(I%)$
  $G(2\cdot N_Z + I%) = X(I%) - 1.0000$
NEXT I%
REM
$T_2 = 0.000$
IF $N_A% > 0$ THEN
  FOR $J% = 1$ TO $N_A$
    $T_2 = T_2 + X(2\cdot N_Z + 1 + J%) \cdot G(A% + J%)$
  NEXT J%
END IF
REM
$F = F + T_2$
REM
END SUB
REM
STOP
END

A1.2 UV intensity programs

A1.2.1 Introduction

The ESSE model developed in Chapter 5 is a versatile model capable of predicting the UV intensity on a surface produced by a UV lamp of known emission power. The model can be re-arranged so that if the UV intensity on a surface is known then the power output of the lamp can be estimated. This is demonstrated in section A1.2.2. Section A1.2.3 details the programs used to calculate the UV intensities on objects of cylindrical geometry. Section A1.2.4 details the programs used to generate the results from Chapter 6 (for the
A1.2.2 Determination of UV lamp power output

(a) Background

The program POWER.FOR is a Fortran 77 program which is used to calculate the power output of a UV lamp. When a UV intensity measurement is known at a given spatial location, it is possible to estimate the power output of the UV lamp that would give such an intensity if the UV lamp's emission characteristics can be described by the ESSE model (see equation 5.5).

The user must provide the UV intensity measurement, \( I \), taken using the UV radiometer at the cylindrical-polar coordinate \((r, \theta, \phi)\). The user must specify the number of point sources which will comprise the UV source by setting the \( N_C \), \( N_L \) and \( N_Z \) parameters (see Figure 5.2). On completion, the program will display the estimate of the power output of the UV lamp.

(b) Program listing

```
C POWER.FOR
C
C Extrene source model employed to provide an estimate of
C the power output of a lamp
C
C By D.Gardner (895)
C
C The model assumes volumetric emission from the lamp.
C
C The program calculates the dimensionless intensity at a point
C specified by the user \((r,\theta,\phi)\), which is merely the sum of the
C dimensionless intensities from the individual point sources
C which are evenly spaced throughout the lamp volume. This program
C is for a non-absorbing medium.
C
REAL*8 S,RL,L1,SP
REAL*8 ISUM,L1,L1,T1,R1,T,PRHOS
REAL*8 RHO,EF,Z1
REAL*8*8 N,X,Y,Y1

INTEGER NL,NC,NP,NZ

PARAMETER (NC=10)
PARAMETER (NL=10)
PARAMETER (NZ=20)
```
Calculate number of point sources

NP=2*NL*NC*NZ.

PI=3.141593

Define cylindrical polar co-ordinates at which UV intensity was determined

R1=(0.08*30.5E-3)
T1=0
L1=41E-3

UV lamp parameters

I=6.29
RL=7.5E-3
LL=0.20

Sum intensities from individual point sources

ISUM=0

DO 100 J=1,NZ
   DO 200 K=1,NC
      DO 300 M=1,2*NL

Calculate cylindrical co-ordinates of the point source

T=(M-1)*PI/NL
R=SQRTR((K-0.5)*RL**2/NC)
L=(J-0.5)*LL/NZ

Calculate the square of the distance between the point source and the point at which the intensity is being determined

RHOS=(R**SIN(T)-R1*SIN(T1))**2
   RHOS=RHOS+(R**COS(T)-R1*COS(T1))**2
   RHOS=RHOS+(L-L1)**2

Calculate cartesian forms

X=R**SIN(T)
   Y=R**COS(T)
   X1=R1**SIN(T1)
   Y1=R1**COS(T1)

Calculate view factors

V1=ATAN(ABS(X1-X)*ABS(Y1-Y))
   V1=COS(V1)

V2=ATAN(ABS(L1-L)*ABS(Y1-Y))
   V2=COS(V2)

ISUM=ISUM+1/RHOS*V1*V2

CONTINUE

300   CONTINUE
200   CONTINUE
100   CONTINUE

SP=1.4*PI/ISUM
S=SP/NP
A1.2.3 UV intensities on objects of cylindrical geometry

(a) Background

Two programs, VERT and VOLTARC, calculate the UV intensities at specified points on the vertical and horizontal surfaces of an object of cylindrical geometry respectively. For both programs only one UV source is specified. The results from these programs are then used by a third program, MULTIVOL, which is used to sum the UV fields produced by individual UV lamps in a multi-lamp system.

The view factors can be calculated by simple geometry. For example, for the vertical surface of a cylinder the view factors, $(\cos \alpha)$ and $(\cos \beta)$, are calculated as follows:

\[
(\cos \beta) = \frac{\Delta x}{\sqrt{(\Delta x)^2 + (\Delta y)^2}}
\]
\[
(\cos \alpha) = \frac{\Delta x}{\sqrt{(\Delta x)^2 + (\Delta z)^2}}
\]

Once the view factors are known then equation (5.7) can be applied.

(b) Program listings

REM VERT.BAS
REM A program to calculate the UV field, produced by 1 Voltarc source
located in the rig, for the side of a cylindrical object.

PARAMETER LIST

PI = 3.141592654

Cylinder

NA% = 1
NP% = 120

Voltarc lamp

NC% = 3
NL% = 5
NZ% = 20

DEFINE INTENSITY STORAGE ARRAY

SDYNAMIC

DIM IO(NA%, NP%)

VARIABLE SET-UP

Lamp parameters

S = 13.1
AL = 0.35
WL = 0.20
RL = 7.5E-3
RD = 0.08

NPS% = 2*NL%*NC%*NZ%
SP = S*WL/(AL*NPS%)

Object parameters

ORAD = 0.033
OH = 0.000
OSZ = 0.00

CLS

LOCATE 1, 1
PRINT "NUMBER OF POINT SOURCES SELECTED ="
LOCATE 1, 36
PRINT NPS%
LOCATE 3, 1
PRINT "NUMBER OF LAMP ZONES ="
LOCATE 3, 24
PRINT NC%
LOCATE 3, 30
PRINT "NUMBER POINTS IN EACH LAMP ZONE ="
LOCATE 3, 65
PRINT NL%*2

LAMP LOOPS - the contribution from each point source comprising the UV source must be taken into account

FOR L% = 1 TO NC%
    FOR M% = 1 TO 2*NL%
        LOCATE 8, 1
        PRINT L%
        LOCATE 8, 10
        PRINT M%

. 197
REM Calculate the radial and theta co-ordinates of the point source
REM
LR=SQR((L%-0.5)*RL^2/NC%)
LT=(M%-1)*PI/NL%
REM
REM Calculate cartesian forms
REM
LX=RDI+LR*COS(LT)
LY=LR*SIN(LT)
REM
REM Establish limits for the object points able to see the UV
REM point source output - calculate theta A and theta I
REM
TA=ATN(LY/LX)
TI=ATN(SQR(LX^2+LY^2-ORAD^2)/ORAD)
REM
REM First OBJECT loop - angular co-ordinate
REM
FOR J%=1 TO NP%
OBT=(J%-1)*2*PI/NP%
REM
REM Calculate object cartesian co-ordinates
REM
OX=ORAD*COS(OBT)
OY=ORAD*SIN(OBT)
REM
REM Calculate delta x and delta y
REM
DX=LX-OX
DY=LY-OY
REM
REM Limit calculations to those object points that receive some
REM UV point source output
REM
IF OBT >=(TA-TI+2*PI) OR OBT <=(TA+T1) THEN
REM
REM Final LAMP LOOP - height co-ordinate
REM
FOR K%=1 TO NZ%
LZ=(K%-0.5)*WNZ%
FOR JO/o=1 TO NA%
IF NA%=I THEN
OBZ=OH
ELSE
OBZ=OZ+(J%-1)*OH/(NA%-1)
END IF
REM
DZ=OBZ-LZ
REM
REM Select object point height
REM
FOR I%=1 TO NA%
IF NA%=I THEN
REM
REM Calculate view factors
REM
VFA=ABS(DX*SQR(DX^2+DZ^2))
VFB=ABS(DX*SQR(DX^2+DY^2))
REM
REM Calculate UV point source contribution to the object point, corrected
REM for the view factors
REM
RHOS=DX^2+DY^2+DZ^2
IO(I%,J%)=IO(I%,J%)+(1/RHOS)*VFA*VFB
REM
NEXT I%
END IF
NEXT M%
NEXT K%
REM
END
REM Multiply IO array through by the constant to obtain actual intensities
REM
IMAX=0
REM
FOR J%=1 TO N%
FOR J%=1 TO NP%
IO(J%,J%)=IO(J%,J%)*SP(^*PI)
IF IO(J%,J%)>IMAX THEN
IMAX=IO(J%,J%)
END IF
NEXT J%
NEXT J%
REM
Save intensity measurements in sequential file
REM
OPEN "INTENSE.DAT" FOR OUTPUT AS #1
WRITE #1, NA%, NP%
FOR Y%=1 TO NA%
FOR Z%=1 TO NP%
WRITE #1, 100YO/Y%,Z%)
NEXT Z%
NEXT Y%
CLOSE #1
OPEN "DIMEN.DAT" FOR OUTPUT AS #1
WRITE #1, NA%, NP%, IMAX
FOR Y%=1 TO NA%
FOR Z%=1 TO NP%
WRITE #1, 100YO/Y%,Z%IMAX
NEXT Z%
NEXT Y%
CLOSE #1
REM
STOP
END

REM VOLTARC.BAS
REM
A program to calculate the UV field produced by 1 Voltarc source located in the rig for a circular disc-shaped object.
REM
REM
REM PARAMETER LIST
REM
PI=3.141592654
REM
Circular disc
REM
NA%=1
NP%=64
REM
Voltarc lamp
REM
NC%=3
N1%=5
NZ%=20
REM
DEFINE INTENSITY STORAGE ARRAY
REM
SDYNAMIC
REM
DIM IO(NA%,NP%)
REM
VARIABLE SET-UP
REM
REM Lamp parameters
REM
S=13.1
AL=0.35
WL=0.20
RL=7.5E-3
RD=0.16
OBZ=0.00
REM
REM NPS%=2*NL%*NC%*NZ%
REM SP=S*WL/(AL*NPS%)
REM
REM Object parameters
REM
REM CLS
LOCATE 1,1
PRINT "NUMBER OF OBJECT POINTS CHOSEN = "
LOCATE 1,134
PRINT NA%*NP%
LOCATE 3,1
PRINT "ANULUSES = "
LOCATE 3,12
PRINT NA%
LOCATE 3,30
PRINT "POINTS IN EACH ANULUS = "
LOCATE 3,56
PRINT NP%
REM
REM OBJECT LOOPS - the UV intensity is calculated for each point on the object
REM top in turn.
REM
FOR I%=1 TO NA%
FOR J%=1 TO NP%
REM Show object point number
REM
LOCATE 8,1
PRINT I%
LOCATE 8,10
PRINT J%
REM
REM Calculate object point cylindrical polar co-ordinates
REM
OBT=(I%/0.5)*2*PI/NP%
OBR=ORAD*(I%/0.5)/NA%
OBR=27E-3
REM
REM Set intensity summation to zero for new object point
REM
ISUM=0
IMAX=0
REM
REM LAMP LOOPS - the contribution from each point source comprising
REM the UV source must be taken into account.
REM
FOR K%=1 TO NZ%
FOR L%=1 TO NC%
FOR M%=1 TO 2*NL%
REM
REM Calculate cylindrical polar co-ordinates of the point source
REM
LT=(M%/1)*PI/NL%
LR=SQR((L%/0.5)*RL/2/NC%)
LL=(K%/0.5)*WL/NZ%
REM
REM Calculate distance from UV point source to point on object
REM
DX=RD+LR*COS(LT)+OBR*COS(OBT)

REM
REM
REM
REM \[ {\text{DY}} = \text{LR} \cdot \text{SIN}(LT) - \text{OBR} \cdot \text{SIN}(OBT) \]
REM \[ {\text{DZ}} = \text{LL} - \text{OBZ} \]
REM
REM \[ \text{RHOS} = \text{DX}^2 + \text{DY}^2 + \text{DZ}^2 \]
REM \[ \text{RHO} = \text{SQR(RHOS)} \]
REM
REM Calculate UV intensity, corrected for the view factor, and add to
REM sum total for the object point
REM
\[ \text{VF} = \text{DZ}/\text{RHO} \]
\[ \text{ISUM} = \text{ISUM} \cdot (1/\text{RHOS}) \cdot \text{VF} \]

REM
REM Complete UV intensity calculation
REM
\[ \text{ISUM} = \text{ISUM} \cdot \text{SP}/(4\pi) \]
REM
REM STORE UV INTENSITY
REM
IF \text{ISUM} > \text{IMAX} THEN
\text{IMAX} = \text{ISUM}
END IF
REM
REM NEXT \text{L}%
REM NEXT \text{K}%
REM
REM Save intensity measurements in sequential file
REM
OPEN "INTENSE.DAT" FOR OUTPUT AS #1
WRITE #1, NA%, NP%
FOR Y%=1 TO NA%
FOR Z%=1 TO NP%
WRITE #1, IO(Y%,Z%)
NEXT Z%
NEXT Y%
CLOSE #1
OPEN "DIMEN.DAT" FOR OUTPUT AS #1
WRITE #1, NA%, NP%, IMAX
FOR Y%=1 TO NA%
FOR Z%=1 TO NP%
WRITE #1, IO(Y%,Z%)/IMAX
NEXT Z%
NEXT Y%
CLOSE #1
REM
REM STOP
END
REM
REM MULTIVOL.BAS - a program to manipulate the data output from
REM VOLTARC.BAS and VERT.BAS in order to model the case of more than one lamp
REM
REM Load the data from VOLTARC.BAS - the sequential file INTENSE.DAT
REM
OPEN "INTENSE.DAT" FOR INPUT AS #1
INPUT #1, NA%, NP%
$DYNAMIC
DIM IO(NA%,NP%)
DIM MIO(NA%,NP%)
FOR I%=1 TO NA%
FOR J%=1 TO NP%
INPUT #1, IO(I%,J%)
NEXT J%
NEXT I%
REM Copy the IO array into MIO

REM FOR I% = 1 TO NAO
  NEXT J%
FOR J% = 1 TO NPO
  MIO(I%, J%) = IO(I%, J%)
NEXT J%
NEXT I%

REM Define the number of lamps
REM NLA%=2
REM NR%=NP%/NLA%
IMAX=0
REM
REM CONVERT THE DATA TO THE SPECIFIED MULTI-LAMP SYSTEM
REM
REM FOR I% = 1 TO NLA%-1
  FOR J% = 1 TO NPO
    C% = J% + NR% * I%
    IF C% > NPO THEN
      C% = C% - NPO
    END IF
    FOR K% = 1 TO NAO
      MIO(K%, C%) = MIO(K%, C%) + IO(K%, J%)
      IF MIO(K%, C%) > IMAX THEN
        IMAX = MIO(K%, C%)
      END IF
    NEXT K%
  NEXT J%
NEXT I%

REM
REM Save the multi-lamp intensity data in INTENSE2.DAT
REM
REM OPEN "INTENSE2.DAT" FOR OUTPUT AS #1
WRITE #1, NAO, NPO
FOR Y% = 1 TO NAO
  FOR Z% = 1 TO NPO
    WRITE #1, MIO(Y%, Z%)
  NEXT Z%
NEXT Y%
CLOSE #1
OPEN "DIMEN.DAT" FOR OUTPUT AS #1
WRITE #1, NAO, NPO, IMAX
FOR Y% = 1 TO NAO
  FOR Z% = 1 TO NPO
    WRITE #1, MIO(Y%, Z%)*IMAX
  NEXT Z%
NEXT Y%
CLOSE #1
REM
REM STOP
END
A1.2.4 UV disinfection tunnel

(a) Background

A computer program, SLABAV, was written to calculate the average UV intensity at specified points on the surface of a slab-shaped object travelling through a ‘tunnel’ of UV lamps. The computer program SLABRES uses the results from SLABAV to determine the survival of micro-organisms on the 6 surfaces of the slab after passing through the disinfection tunnel in a specified time, T. (See section 6.2.2 for further information).

(b) Program listings

REM
REM SLABAV.BAS
REM
REM A program to calculate the UV intensity profile on an object
REM of slab geometry within a tunnel-shaped chamber of UV lamps.
REM
REM Parameter set-up
REM
REM UV lamp parameters
REM
S=13.1 'power output from one UV lamp /W
LL=0.33 'length of the UV lamp /m
RL=7.5E-3 'radius of the UV lamp /m

REM
NC%=3 'parameters to divide the UV lamp up into finite
NL%=2 'zones. At the centre of each finite zone there
NZ%=10 'is a point UV source.

REM
DIM UVP%0(NUVL%) 'number of UV lamps
REM
DIM UVX(NUVL%) 'flag to determine whether the UV lamp is suspended
REM 'vertically (0) or horizontally (1)
REM
DIM UVX(NUVL%) 'cartesian co-ordinates of the top end of the UV lamps
DIM UVY(NUVL%) ' (or end with lower x co-ordinate in the case of
DIM UVZ(NUVL%) ' horizontal lamps)

REM
Note: x = distance from start of tunnel
REM
y = distance from wall
REM
z = height above the floor
REM
RESTORE 1000
FOR X%=1 TO NUVL%
REM
READ UVP%(X%)
READ UVX(X%)
READ UVY(X%)
READ UVZ(X%)
NEXT X%
REM
NP%=2*NC%*NL%*NZ% 'number of point sources comprising 1 UV lamp
REM

203
SP=S/NP% 'power output of one point UV source

PI=3.141593

Slab parameters

SLABH = 2.00 'slab height /m (x dimension)
SLABW = 0.25 'slab width /m (x dimension)
SLABB = 0.25 'slab breadth /m (y dimension)

Define the number of locations along the tunnel at which the surface intensities are to be calculated.

NTUN% = 10 'number of positions along the tunnel at which the UV intensity is to be calculated.
NL11% = 10 'number of points in length of influence #1 (before tunnel)
NL12% = 10 'number of points in length of influence #2 (after tunnel)

NPOS% = NTUN% + NL11% + NL12% 'number of positions at which intensity is calculated

LTUN = 1.000 'length of repeatable section of tunnel
LENPOS = LTUN/(NTUN%) 'length between positions in the tunnel

Define the number of points on each slab surface at which the UV intensity is to be calculated.

N1Z% = 9
N1X% = 9
N2Z% = 9
N2X% = 9
N3Z% = 9
N3Y% = 9
N4Z% = 9
N4Y% = 9
N5X% = 9
N5Y% = 9
N6X% = 9
N6Y% = 9

Define the arrays containing the UV intensity profiles for each of the 6 surfaces of the slab

DIM S1(NPOS%,N1Z%,N1X%) 'surface 1
DIM S2(NPOS%,N2Z%,N2X%) 'surface 2
DIM S3(NPOS%,N3Z%,N3Y%) 'surface 3
DIM S4(NPOS%,N4Z%,N4Y%) 'etc.
DIM S5(NPOS%,N5X%,N5Y%)
DIM S6(NPOS%,N6X%,N6Y%)

Specify the cartesian co-ordinates of the centre of the slab

SCXB = -(NL11% - 0.5)*LENPOS 'beginning x co-ordinate of slab centre
SCXE = LTUN + (NL12% - 0.5)*LENPOS 'end x co-ordinate of slab centre
SCY = 1.00 'y co-ordinate
SCZ = 1.00 'z co-ordinate

Main loop - determine slab location in the tunnel

SCREEN 0
CLS
LOCATE 1,1
PRINT "Slab position no."
LOCATE 1,25
PRINT "/",NPOS 
REM
FOR P%=1 TO NPOS 
REM
LOCATE 1,20
PRINT USING "/",P%
REM
SCX = SCXB + (P%-1)*LENPOS
REM
REM
Pick each surface point and calculate the UV intensity at it.
REM
REM
Surface 1
REM
REM
IAV=0.0000
REM
FOR I%=1 TO N1Z%
FOR J%=1 TO N1X%
REM
State surface point co-ordinates
REM
SX=(I%-0.5)*SLABWN1X%-SLABW/2 + SCX
SY=SCY - SLABB/2
SZ=(I%-0.5)*SLABH/N1Z% + SCZ + SLABH/2
REM
SURF%=1
ISUM=0.0000
REM
CALL INTENSE(SURF%,SX,SY,SZ,ISUM)
REM
S1(P%,I%,J%) = ISUM
REM
NEXT J%
NEXT I%
REM
REM
REM
Surface 2
REM
REM
IAV=0.0000
REM
FOR I%=1 TO N2Z%
FOR J%=1 TO N2X%
REM
SX=(I%-0.5)*SLABWN2X%-SLABW/2 + SCX
SY=SCY - SLABB/2
SZ=SCZ + SLABB/2 - (I%-0.5)*SLABH/N2Z%
REM
SURF%=2
ISUM=0.0000
REM
CALL INTENSE(SURF%,SX,SY,SZ,ISUM)
REM
S2(P%,I%,J%) = ISUM
REM
NEXT J%
NEXT I%
REM
REM
REM
Surface 3
REM
REM
IAV=0.0000
REM
FOR I%=1 TO N3Z%
FOR J%=1 TO N3Y%
REM
SX=SCX - SLABW/2
SY=SCY + SLABB/2 - (I%-0.5)*SLABB/N3Y%
SZ=SCZ + SLABH/2 - (I%-0.5)*SLABH/N3Z%
REM
SURF%=3
ISUM=0.0000
REM

205
CALL INTENSE(SURF%, SX, SY, SZ, ISUM)
\[ S3(P\%_4, I\%, J\%) = ISUM \]
NEXT J%
REM
Surface 4
REM
IAV = 0.00000
REM
FOR I% = 1 TO N4Z%
FOR J% = 1 TO N4Y%
SX = SCX + SLABW/2
SY = SCY - SLABB/2 + (J% - 0.5) * SLABB/N4Y%
SZ = SCZ + SLABH/2 - (J% - 0.5) * SLABH/N4Z%
REM
SURF% = 4
ISUM = 0.00000
REM
CALL INTENSE(SURF%, SX, SY, SZ, ISUM)
\[ S4(P\%, I\%, J\%) = ISUM \]
NEXT J%
REM
REM
Surface 5
REM
IAV = 0.00000
REM
FOR I% = 1 TO N5X%
FOR J% = 1 TO N5Y%
SX = SCX - SLABW/2 - (I% - 0.5) * SLABW/N5X%
SY = SCY - SLABB/2 + (I% - 0.5) * SLABB/N5Y%
SZ = SCZ + SLABH/2
REM
SURF% = 5
ISUM = 0.00000
REM
CALL INTENSE(SURF%, SX, SY, SZ, ISUM)
\[ S5(P\%, I\%, J\%) = ISUM \]
NEXT J%
REM
REM
Surface 6
REM
IAV = 0.00000
REM
FOR I% = 1 TO N6X%
FOR J% = 1 TO N6Y%
SX = SCX + SLABW/2 - (I% - 0.5) * SLABW/N6X%
SY = SCY - SLABB/2 + (I% - 0.5) * SLABB/N6Y%
SZ = SCZ - SLABH/2
REM
SURF% = 6
ISUM = 0.00000
REM
CALL INTENSE(SURF%, SX, SY, SZ, ISUM)
\[ S6(P\%, I\%, J\%) = ISUM \]
NEXT J%
REM
NEXT P%
Write results to sequential files

SCREEN 0
CLS

LOCATE 1,1
PRINT "Calculations complete - writing sequential files containing results"

OPEN "SURF1.DAT" FOR OUTPUT AS #1
REM Surface 1
REM
WRITE #1,NPOS%
WRITE #1,NTUN%
WRITE #1,NL11%
WRITE #1,NL12%
WRITE #1,LTUN
WRITE #1,LENPOS
REM
WRITE #1,NIZ%
WRITE #1,NIX%
REM
FOR P%=1 TO NPOS%
  FOR I%=1 TO NIZ%
    FOR J%=1 TO NIX%
      WRITE #1,S1(P%,I%,J%)
    NEXT
  NEXT
NEXT
REM
CLOSE #1
REM
Surface 2
REM
OPEN "SURF2.DAT" FOR OUTPUT AS #1
REM
WRITE #1,NPOS%
WRITE #1,NTUN%
WRITE #1,NL11%
WRITE #1,NL12%
WRITE #1,LTUN
WRITE #1,LENPOS
REM
WRITE #1,NIZ%
WRITE #1,NIX%
REM
FOR P%=1 TO NPOS%
  FOR I%=1 TO NIZ%
    FOR J%=1 TO NIX%
      WRITE #1,S2(P%,I%,J%)
    NEXT
  NEXT
NEXT
REM
CLOSE #1
REM
Surface 3
REM
OPEN "SURF3.DAT" FOR OUTPUT AS #1
REM
WRITE #1,NPOS%
WRITE #1,NTUN%
WRITE #1,NL11%
WRITE #1,NL12%
WRITE #1,LTUN
WRITE #1,LENPOS
REM WRITE #1,N32%
WRITE #1,N33Y%
REM FOR P%=1 TO NPOS%
  FOR J%=1 TO N32%
    FOR J%=1 TO N3Y%
      WRITE #1,S3(P%,J%,J%)%
    NEXT J%
  NEXT J%
REM
REM OPEN "SURF4.DAT" FOR OUTPUT AS #1
REM WRITE #1,NPOS%
WRITE #1,NTUN%
WRITE #1,NL11%
WRITE #1,NL12%
WRITE #1,LTUN
WRITE #1,LENPOS
REM WRITE #1,N42%
WRITE #1,N4Y%
REM FOR P%=1 TO NPOS%
  FOR J%=1 TO N42%
    FOR J%=1 TO N4Y%
      WRITE #1,S4(P%,J%,J%)%
    NEXT J%
  NEXT J%
REM
REM CLOSE #1
REM
REM Surface 4
REM
REM OPEN "SURF5.DAT" FOR OUTPUT AS #1
REM WRITE #1,NPOS%
WRITE #1,NTUN%
WRITE #1,NL11%
WRITE #1,NL12%
WRITE #1,LTUN
WRITE #1,LENPOS
REM WRITE #1,N5X%
WRITE #1,N5Y%
REM FOR P%=1 TO NPOS%
  FOR J%=1 TO N5X%
    FOR J%=1 TO N5Y%
      WRITE #1,S5(P%,J%,J%)%
    NEXT J%
  NEXT J%
REM
REM CLOSE #1
REM
REM Surface 5
REM
REM OPEN "SURF6.DAT" FOR OUTPUT AS #1
REM WRITE #1,NPOS%
WRITE #1,NTUN%
WRITE #1,NL11%
WRITE #1,NL12%
WRITE #1,LTUN
WRITE #1,LENPOS

REM
WRITE #1,N6X%
WRITE #1,N6Y%

REM
FOR P%=1 TO NPOS%
  FOR I%=1 TO N6X%
    FOR J%=1 TO N6Y%
      WRITE #1,S6(P%,I%,J%)
    NEXT J%
  NEXT I%
NEXT P%

REM
CLOSE #1

REM
Display results

REM
LOCATE 5,1
PRINT "The results have been generated and stored in the sequential files:"
PRINT PRINT "SURF1.DAT - SURF6.DAT for the 6 surfaces of the slab" LOCATE 15,1
INPUT "Hit enter to stop ";$S
STOP

REM
Cartesian co-ordinates of UV lamps

REM
1000 DATA 0,0.500,0.000,0.175 ' UV lamp #1 (vertical)
DATA 0,0.500,0.000,2.175
DATA 0,0.500,2.000,0.175
DATA 0,0.500,2.000,2.175

REM
SUB INTENSE(SURF%,SX,SY,SZ,ISUM)

REM
SHARED NUVL%,NC%,NL%,NZ%,PLL,SP,UVX0,UVY0,UVZ0
SHARED UPV%()

REM
ISUM=0.00000

REM
FOR A%=1 TO NUVL% ' UV lamp loop
  FOR J%=1 TO NZ%
    FOR K%=1 TO NC%
      FOR M%=1 TO 2*NL%
        T=(M%-1)*PI/NL%
        R=SQR((K%-0.5)*RL^2/NC%)
        L=(J%-0.5)*PLL/NZ%
        X=UVX(A%)+L
        Y=UVY(A%)+R*SIN(T)
        Z=UVZ(A%)+R*COS(T)
      NEXT M%
    NEXT K%
  NEXT J%
NEXT A%

REM
Calculate the local cylindrical-polar co-ordinates of the UV point source
(r,l,theta)

REM
(t,r,l)

REM
Convert cylindrical-polar co-ordinates for the UV point source into cartesian co-ordinates. (X,Y,Z)

REM
IF UPV%0 THEN 'vertical lamp
  X=UVX(A%) - RL*COS(T)
  Y=UVY(A%) + RL*SIN(T)
  Z=UVZ(A%) - L
ELSE 'horizontal lamp
  X=UVX(A%) + L
  Y=UVY(A%) - RL*SIN(T)
  Z=UVZ(A%) + RL*COS(T)
END IF

209
REM Calculate the square of the distance between the UV point source (X,Y,Z) and the point on the surface of the slab (SX,SY,SZ)

REM Calculate the view factors

RHOS=(X-SX)^2 + (Y-SY)^2 + (Z-SZ)^2

SELECT CASE SURF%
CASE 1
IF Y<SY THEN
T2=SQR((X-SX)^2+(Y-SY)^2)
T1=ABS(Y-SY)
COSAL=T1/T2
T2=SQR((Y-SY)^2+(Z-SZ)^2)
COSBE=T1/T2
ELSE
COSAL=0.0000
COSBE=0.0000
END IF
CASE 2
IF Y>SY THEN
T2=SQR((X-SX)^2+(Y-SY)^2)
T1=ABS(Y-SY)
COSAL=T1/T2
T2=SQR((Y-SY)^2+(Z-SZ)^2)
COSBE=T1/T2
ELSE
COSAL=0.0000
COSBE=0.0000
END IF
CASE 3
IF X<SX THEN
T1=ABS((X-SX))
T2=SQR((X-SX)^2+(Y-SY)^2)
COSAL=T1/T2
T2=SQR((Y-SY)^2+(Z-SZ)^2)
COSBE=T1/T2
ELSE
COSAL=0.0000
COSBE=0.0000
END IF
CASE 4
IF X>SX THEN
T1=ABS((X-SX))
T2=SQR((X-SX)^2+(Y-SY)^2)
COSAL=T1/T2
T2=SQR((Y-SY)^2+(Z-SZ)^2)
COSBE=T1/T2
ELSE
COSAL=0.0000
COSBE=0.0000
END IF
CASE 5
IF Z>SZ THEN
T1=ABS(Y-SY)
T2=SQR((X-SX)^2+(Y-SY)^2)
COSAL=T1/T2
CASE 6

IF Z<SZ THEN
   T1=ABS(Z-SZ)
   T2=SQR((Y-SY)**2+(Z-
   COSBE=T1/T2
   COSAL=0.0000
   COSBE=0.0000
   END IF

ELSE

   IF Z<SY THEN
      T1=ABS(Y-SY)
      T2=SQR((X-SX)**2+(Y-
      COSAL=T1/T2
      T1=ABS(Z-SZ)
      T2=SQR((Y-SY)**2+(Z-
      COSBE=T1/T2
      COSAL=0.0000
      COSBE=0.0000
      END IF

END SELECT

ISUM=ISUM+I/RHOS*COSAL*COSBE

NEXT M%

NEXT A%

REM Take the power output of the lamp into account
REM
ISUM=ISUM*SP/(4*PI)
REM
END SUB
REM
END

REM
SLABRES.BAS
REM
A program to convert the intensity at the surfaces of a slab into
average UV intensity, fluence and disinfection level given a velocity
for the slab through the tunnel.
REM
$DYNAMIC
REM
DIM DISINF(6) 'disinfection per side
REM
TOTDISF = 0.000 'total disinfection level for the slab
REM
REM Slab dimensions
REM
SLABH = 2.00 'slab height / m (z dimension)
SLABW = 0.25 'slab width / m (x dimension)
SLABB = 0.25 'slab breadth / m (y dimension)
REM
REM Calculate the fraction of the total surface area of the slab that
REM each surface of the slab contributes.
REM
A1 = SLABW * SLABH
A2 = SLABW * SLABH
A3 = SLABB * SLABH
A4 = SLABB * SLABH
A5 = SLABB * SLABW
A6 = SLABB * SLABW

REM
SAREA = A1 + A2 + A3 + A4 + A5 + A6
REM
A1 = A1 / SAREA
A2 = A2 / SAREA
A3 = A3 / SAREA
A4 = A4 / SAREA
A5 = A5 / SAREA
A6 = A6 / SAREA

REM
CLS
PRINT "COMMENCING CALCULATIONS"
REM
REM Load the results from SLABAV.BAS
REM
REM Surface 1
REM
OPEN "SURF1.DAT" FOR INPUT AS #1
REM
INPUT #1,NPOS%
INPUT #1,NTUN%
INPUT #1,NLI1%
INPUT #1,NLI2%
INPUT #1,LTUN
INPUT #1,LENPOS
REM
INPUT #1,NIZ%
INPUT #1,NIX%
REM
DIM S1(NPOS%,N1Z%,N1X%)
DIM SAV1(N1Z%,N1X%)
DIM D1(N1Z%,N1X%)
REM
FOR P%=1 TO NPOS%
  FOR 10/o=1 TO N1Z%
    FOR J%=1 TO N1X%
      INPlT #1,S1(P%,10/o,J%)
    NEXT J%
  NEXT 10/o
NEXT P%
REM
CLOSE #1
REM
Surface 2
REM
OPEN "SURF2.DAT" FOR INPUT AS #1
REM
INPUT #1,NPOS%
INPUT #1,NTUN%
INPUT #1,NLI1%
INPUT #1,NLI2%
INPUT #1,LTUN
INPUT #1,LENPOS
REM
INPUT #1,N2Z%
INPUT #1,N2X%
REM
DIM S2(NPOS%,N2Z%,N2X%)
DIM SAV2(N2Z%,N2X%)
DIM D2(N2Z%,N2X%)
REM
FOR P%=1 TO NPOS%
  FOR 1%=1 TO N2Z%
    FOR J%=1 TO N2X%
      INPlT #1,S1(P%,1%,J%)
    NEXT J%
  NEXT 1%=1 TO N2X%
INPUT #1, S2(P%, I%, J%)
NEXT J%
NEXT P%
REM
CLOSE #1
REM
DIM S3(NPOS%, N3Z%, N3Y%)
DIM SAV3(N3Z%, N3Y%)
DIM D3(N3Z%, N3Y%)
REM
FOR P%=1 TO NPOS%
FOR I%=1 TO N3Z%
FOR J%=1 TO N3Y%
INPUT #1, S3(P%, I%, J%)
NEXT J%
NEXT I%
NEXT P%
REM
DIM S4(NPOS%, N4Z%, N4Y%)
DIM SAV4(N4Z%, N4Y%)
DIM D4(N4Z%, N4Y%)
REM
FOR P%=1 TO NPOS%
FOR I%=1 TO N4Z%
FOR J%=1 TO N4Y%
INPUT #1, S4(P%, I%, J%)
NEXT J%
NEXT I%
NEXT P%
REM
CLOSE #1
REM
DIM S5(NPOS%, N5Z%, N5Y%)
DIM SAV5(N5Z%, N5Y%)
DIM D5(N5Z%, N5Y%)
REM
FOR P%=1 TO NPOS%
FOR I%=1 TO N5Z%
FOR J%=1 TO N5Y%
INPUT #1, S5(P%, I%, J%)
NEXT J%
NEXT I%
NEXT P%
REM
CLOSE #1
REM
DIM S6(NPOS%, N6Z%, N6Y%)
DIM SAV6(N6Z%, N6Y%)
DIM D6(N6Z%, N6Y%)
REM
FOR P%=1 TO NPOS%
FOR I%=1 TO N6Z%
FOR J%=1 TO N6Y%
INPUT #1, S6(P%, I%, J%)
NEXT J%
NEXT I%
NEXT P%
DATA INPUT #1,NPOS%
DATA #1,NTUN%
DATA #1,NLI1%
DATA #1,NLI2%
DATA #1,LTUN
DATA #1,LENPOS

DATA INPUT #1,N5X%
DATA #1,N5Y%

DATA DIM SS(NPOS%,N5X%,N5Y%)
DATA DIM DAV(NPOS%,N5Y%)
DATA DIM D5(N5X%,N5Y%)

REM FOR P%=1 TO NPOS%
  FOR I%=1 TO N5X%
    FOR J%=1 TO N5Y%
      INPUT #1,SS(P%,I%,J%)
    NEXT J%
  NEXT I%
NEXT P%

REM CLOSE #1

REM Surface 6

REM OPEN "SURF6.DAT" FOR INPUT AS #1

REM DATA INPUT #1,NPOS%
DATA #1,NTUN%
DATA #1,NLI1%
DATA #1,NLI2%
DATA #1,LTUN
DATA #1,LENPOS

REM DATA INPUT #1,N6X%
DATA #1,N6Y%

REM DATA DIM S6(NPOS%,N6X%,N6Y%)
DATA DIM DAV(N6X%,N6Y%)
DATA DIM D6(N6X%,N6Y%)

REM FOR P%=1 TO NPOS%
  FOR I%=1 TO N6X%
    FOR J%=1 TO N6Y%
      INPUT #1,S6(P%,I%,J%)
    NEXT J%
  NEXT I%
NEXT P%

REM CLOSE #1

REM Data now loaded. Start converting.

REM OPEN "DISINF1.DAT" FOR OUTPUT AS #1
OPEN "DISINF2.DAT" FOR OUTPUT AS #2
OPEN "DISINF3.DAT" FOR OUTPUT AS #3
OPEN "DISINF4.DAT" FOR OUTPUT AS #4
OPEN "DISINF5.DAT" FOR OUTPUT AS #5
OPEN "DISINF6.DAT" FOR OUTPUT AS #6
OPEN "DISINF7.DAT" FOR OUTPUT AS #7

REM Define the residence times at which survival to be calculated
REM FOR T = 0.000 TO 2000 STEP 20

REM Calculate the average UV intensity
REM Surface 1
REM DISINFS(1) = 0.000
REM FOR I%=1 TO N1Z%
   FOR J%=1 TO N1X%
      SAV1(I%,J%) = 0.0000
      FOR P%=1 TO NPOS%
         SAV1(I%,J%) = SAV1(I%,J%) + S1(P%,I%,J%)
      NEXT P%
   NEXT J%
REM SAV1(I%,J%) = SAV1(I%,J%) / NPOS%
REM Have calculated the average UV intensity per surface point through
REM the tunnel. Now calculate the disinfection per surface point
REM achieved in a trip through a specified number of repeatable sections
REM at a given velocity.
REM CALL DISINF(SAV1(%,J%),DI(J%,J%))
REM DISINFS(1) = DISINFS(1) + DI(%,J%)
NEXT J%
REM NEXT I%
REM Calculate the disinfection (survival) level for side 1
REM assuming an even initial microbial load
REM DISINFS(1) = DISINFS(1) / (N1Z% * N1X%)
REM Add to running total of disinfection level for the whole slab
REM TOTDISF = TOTDISF + DISINFS(1) * A1
REM Surface 2
REM DISINFS(2) = 0.000
REM FOR I%=1 TO N2Z%
   FOR J%=1 TO N2X%
      SAV2(I%,J%) = 0.0000
      FOR P%=1 TO NPOS%
         SAV2(I%,J%) = SAV2(I%,J%) + S2(P%,I%,J%)
      NEXT P%
   NEXT J%
REM SAV2(I%,J%) = SAV2(I%,J%) / NPOS%
REM Have calculated the average UV intensity per surface point through
REM the tunnel. Now calculate the disinfection per surface point
REM achieved in a trip through a specified number of repeatable sections
REM at a given velocity.
REM CALL DISINF(SAV2(I%,J%),D2(I%,J%))
REM DISINFS(2) = DISINFS(2) + D2(I%,J%)
NEXT J%
REM NEXT I%
REM DISINFS(2) = DISINFS(2) / (N2Z% * N2X%)
REM TOTDISF = TOTDISF + DISINFS(2) * A2
REM Surface 3
REM DISINFS(3) = 0.000
REM
FOR 1%=1 TO N3Z%
  FOR J%=1 TO N3Y%
    SAV3(1%,J%) = 0.0000
  NEXT P%
END REM

REM SAV3(1%,J%) = SAV3(1%,J%) / NPOS%
REM
REM Have calculated the average UV intensity per surface point through
REM the tunnel. Now calculate the disinfection per surface point
REM achieved in a trip through a specified number of repeatable sections
REM at a given velocity.
REM
REM CALL DISINF(SAV3(1%,J%), D3(1%,J%))
REM
REM DISINFS(3) = DISINFS(3) + D3(1%,J%)
REM
NEXT J%
REM
REM DISINFS(3) = DISINFS(3) / (N3Z% * N3Y%)
REM
REM TOTDISF = TOTDISF + DISINFS(3) * A3
REM
REM Surface 4
REM
REM DISINFS(4) = 0.000
REM
FOR 1%=1 TO N4Z%
  FOR J%=1 TO N4Y%
    SAV4(1%,J%) = 0.0000
  NEXT P%
END REM

REM SAV4(1%,J%) = SAV4(1%,J%) / NPOS%
REM
REM Have calculated the average UV intensity per surface point through
REM the tunnel. Now calculate the disinfection per surface point
REM achieved in a trip through a specified number of repeatable sections
REM at a given velocity.
REM
REM CALL DISINF(SAV4(1%,J%), D4(1%,J%))
REM
REM DISINFS(4) = DISINFS(4) + D4(1%,J%)
REM
NEXT J%
REM
REM DISINFS(4) = DISINFS(4) / (N4Z% * N4Y%)
REM
REM TOTDISF = TOTDISF + DISINFS(4) * A4
REM
REM Surface 5
REM
REM DISINFS(5) = 0.000
REM
FOR 1%=1 TO N5Z%
  FOR J%=1 TO N5Y%
    SAV5(1%,J%) = 0.0000
  NEXT P%
END REM

REM SAV5(1%,J%) = SAV5(1%,J%) / NPOS%
REM

REM.
REM Have calculated the average UV intensity per surface point through the tunnel. Now calculate the disinfection per surface point achieved in a trip through a specified number of repeatable sections at a given velocity.
REM
CALL DISINF(SAV5(1%,J%),D5(1%,J%))
REM
DISINFS(5) = DISINFS(5) + D5(1%,J%)
REM
NEXT J%
NEXT 1%
REM
DISINFS(5) = DISINFS(5) / (N5X% * N5Y%)
REM
TOTDISF = TOTDISF + DISINFS(5) * A5
REM
REM
TOUR6 = 0.000
REM
FOR 1%=1 TO N6X%
FOR J%=1 TO N6Y%
SAV6(1%,J%) = 0.0000
FOR P%=1 TO NPOS%
SAV6(1%,J%) = SAV6(1%,J%) + S6(P%,1%,J%)
NEXT P%
REM
SAV6(1%,J%) = SAV6(1%,J%) / NPOS%
REM
REM Have calculated the average UV intensity per surface point through the tunnel. Now calculate the disinfection per surface point achieved in a trip through a specified number of repeatable sections at a given velocity.
REM
CALL DISINF(SAV6(I%,J%),D6(I%,J%))
REM
DISINFS(6) = DISINFS(6) + D6(I%,J%)
REM
NEXT J%
NEXT 1%
REM
DISINFS(6) = DISINFS(6) / (N6X% * N6Y%)
REM
TOTDISF = TOTDISF + DISINFS * A6
REM
REM
NOW WRITE THE RESULTS TO SEQUENTIAL FILES
REM
WRITE #1,LOG10(DISINFS(1))
WRITE #2,LOG10(DISINFS(2))
WRITE #3,LOG10(DISINFS(3))
WRITE #4,LOG10(DISINFS(4))
WRITE #5,LOG10(DISINFS(5))
WRITE #6,LOG10(DISINFS(6))
WRITE #7,LOG10(TOTDISF)
REM
NEXT T
REM
CLOSE #1
CLOSE #2
CLOSE #3
CLOSE #4
CLOSE #5
CLOSE #6
CLOSE #7
REM
OPEN "SURFAV1.DAT" FOR OUTPUT AS #1
OPEN "SURFAV2.DAT" FOR OUTPUT AS #2
OPEN "SURFAV1.DAT" FOR OUTPUT AS #3
OPEN "SURFAV4.DAT" FOR OUTPUT AS #4
OPEN "SURFAV5.DAT" FOR OUTPUT AS #5
OPEN "SURFAV6.DAT" FOR OUTPUT AS #6

REM
FOR I%=1 TO N1Z%
    FOR J%=1 TO N1X%
        WRITE #1,SAV1(I%,J%)
        WRITE #2,SAV2(I%,J%)
        WRITE #3,SAV3(I%,J%)
        WRITE #4,SAV4(I%,J%)
        WRITE #5,SAV5(I%,J%)
        WRITE #6,SAV6(I%,J%)
    NEXT J%
NEXT I%

REM
CLOSE #1
CLOSE #2
CLOSE #3
CLOSE #4
CLOSE #5
CLOSE #6

REM
LOCATE 10,1
PRINT "CALCULATIONS COMPLETE AND RESULTS WRITTEN TO FILES"

REM
STOP
REM Calculate the fractional survival per point for a specified UV intensity.
REM
REM SUB DISINF(IAV,YAV)
REM
SHARED LTUN,T
REM IAV = average UV intensity
REM FAV = average UV fluence
REM YAV = average microbial survival (fractional - NOT in log form)
REM
REM Specify the slab velocity through the tunnel
REM
V = 0.20 velocity / m/s
REM
REM RT = LTUN / V  residence time in a repeatable section
REM of the tunnel.
REM
REM Define the number of repeatable units which comprise the tunnel.
REM
NREPEAT% = 10
REM
REM First calculate the average survival. Then correct for surface shielding
REM if specified.
REM
FAV = IAV * T
REM
REM Use surface shielding function.
REM
YAV = YACT(FAV)
REM
REM END SUB
REM
FUNCTION YACT(FAV)
REM This function gives the actual disinfection level, YACT, for a given surface fluence, FAV, when surface shielding parameters are specified.
REM
218
Unshielded kinetic parameters - 1st order kinetics (UV only)

\[ K = 2.13 \times 10^{-2} \]

Unshielded kinetic parameters - 1st order kinetics (UV + 1.0% H2O2)

\[ K1 = 0.129 \]

Surface shielding parameters

NZ\% = 1

\[ \text{DIM FR(NZ\%)} \] 'fraction of m\%/in each zone

\[ \text{DIM AL(NZ\%)} \] 'shielding factors in each zone

\[ \text{FR(1)} = 1.000 \]
\[ \text{FR(2)} = 0.2291 \]
\[ \text{FR(3)} = 0.3449 \]

\[ \text{AL(1)} = 1.000 \]
\[ \text{AL(2)} = 0.2784 \]
\[ \text{AL(3)} = 0.01378 \]

\[ \text{YAC1} = 0.000 \]

FOR J\% = 1 TO NZ\%

Calculate the survival in this zone, YZONE, for the actual fluence

\[ \text{FACT} = FAV \times AL(J\%) \]

Define the fraction of coverage of the surface by 1% H2O2 spray

\[ \text{COV} = 1.00 \]

\[ \text{YZONE} = (1.0 - COV) \times \exp(-K1*FACT) \]
\[ \text{YZONE} = YZONE + COV \times \exp(-K1*FACT) \]

Add the contribution from this zone to the overall survival

\[ \text{YAC1} = YAC1 + FR(J\%) \times YZONE \]

NEXT J\%

YACT = YAC1

END FUNCTION

STOP

END
APPENDIX 2. IMPACT TESTS IN THE SPRAY CHAMBER

A2.1 Introduction

It was seen in Chapter 3 that an increase in the disinfection rate could be obtained if the spray was electrostatically-charged. It was proposed that this was due to an increase in the deposition of hydrogen peroxide spray on the test object. In order to investigate this proposition, impact tests were performed with electrostatically-charged sprays so as to directly measure the mass of spray deposited on the test object. The term 'impact tests' refers to the measurement of the mass of spray deposited on the object. Two methods of quantifying the mass of spray deposited on an object's surfaces were developed (see section 2.7). These methods involved collecting the spray deposited on the object in filter paper strips and determining the mass deposited using a precision balance.

A2.2 Impact rate

The spray impact rate on the test object when the spray was not charged was investigated first. Figures A2.1 - A2.3 show the mass of spray deposited on the top of the cylinder and disc objects when placed inside the spray chamber and exposed to an atomised spray of distilled water for various periods of time. Figure A2.2 shows the impact rate under identical conditions to those employed in the disinfection experiments detailed in Chapter 3. These figures show that the impact rate of spray on the top surfaces of the objects was constant over the duration of the experiments and for the particular arrangement employed.
FIGURE A2.1 Spray impact on the top of the PVC cylinder (height = 10 cm, diameter = 4 cm)
(Impact rate = 0.138 mg cm\(^{-2}\) s\(^{-1}\), Spray rate = 0.30 ml s\(^{-1}\))
FIGURE A2.2  Spray impact rate on top of disc object ( height = 2 cm , diameter = 6.6 cm ) under disinfection experimental conditions  
(Impact rate = 0.0251 mg cm$^{-2}$ s$^{-1}$, Spray rate = 0.30 ml s$^{-1}$)
FIGURE A2.3  Spray impact on top of the disc object (height = 2 cm, diameter = 6.6 cm)

( Impact rate = 0.0427 mg cm\(^{-2}\) s\(^{-1}\), Spray rate = 0.23 ml s\(^{-1}\) )
A2.3 Electrostatic spraying

It was previously shown that the use of electrostatic sprays produced an increased disinfection performance (see section 3.6). In order to establish whether this could be attributed to increased deposition of spray on the object, impact tests were undertaken in the spray chamber using distilled water. Three objects were used in these studies - the PVC cylinder (hollow bottom), the PVC disc and the stainless steel hollow cylinder.

It was found that the highest potential that the nozzle could be raised to before a route to earth was created and charge lost (causing 'tripping' of the power supply) was about -16 kV. At this potential short-circuiting paths must have formed, probably by ionising the air in the spray chamber which allowed a circuit to be created from the nozzle through the air, through the QVF body of the spray chamber and finally through the support frame to earth. To avoid this the nozzle was charged to the highest potential (-15 kV) which resulted in stable operation.

Table A2.1 shows the quantity of spray deposited on filter paper strips attached to the side of the stainless steel cylindrical object. Three experiments were performed on two separate days and the table shows the average mass of spray deposited on the filter paper strips. The results show that by raising the nozzle to -15 kV potential, the mass of spray deposited was between 2.3 and 2.7 times greater than for an uncharged nozzle.

The results for the stainless steel and PVC cylinders are shown in Table A2.2. Whilst increased deposition of spray was observed for both objects when the nozzle was charged, the increase in spray deposition was much smaller than had previously been obtained (Table A2.1). The data in Table A2.2 shows only a 10-15% increase in deposition compared to the 130-170% increase in deposition shown in Table A2.1.
Experiment 0 kV NOZZLE POTENTIAL -15 kV NOZZLE POTENTIAL

<table>
<thead>
<tr>
<th></th>
<th>0 kV NOZZLE</th>
<th>POTENTIAL</th>
<th>-15 kV NOZZLE</th>
<th>POTENTIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average mass gained / mg cm(^2) (A)</td>
<td>Range</td>
<td>Average mass gained / mg cm(^2) (B)</td>
<td>Range</td>
</tr>
<tr>
<td>1</td>
<td>2.2</td>
<td>+/- 11%</td>
<td>5.0</td>
<td>+/- 17%</td>
</tr>
<tr>
<td>2</td>
<td>2.3</td>
<td>+/- 21%</td>
<td>6.3</td>
<td>+/- 19%</td>
</tr>
</tbody>
</table>

Spray parameters: 30 seconds spray pulse, 1.5 bar feed air pressure

**TABLE A2.1** Effect of electrostatically-charging the spray on the impact rate on the outside of the stainless steel hollow cylindrical object

<table>
<thead>
<tr>
<th>Object</th>
<th>Surface</th>
<th>Nozzle potential / kV</th>
<th>Mass gained / mg cm(^2)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC CYLINDER</td>
<td>SIDE</td>
<td>0</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>PVC CYLINDER</td>
<td>SIDE</td>
<td>-15</td>
<td>1.34</td>
<td>1.13</td>
</tr>
<tr>
<td>PVC CYLINDER</td>
<td>TOP</td>
<td>0</td>
<td>3.23</td>
<td></td>
</tr>
<tr>
<td>PVC CYLINDER</td>
<td>TOP</td>
<td>-15</td>
<td>3.56</td>
<td>1.10</td>
</tr>
<tr>
<td>STAINLESS STEEL CYLINDER</td>
<td>SIDE</td>
<td>0</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>STAINLESS STEEL CYLINDER</td>
<td>SIDE</td>
<td>15</td>
<td>1.35</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Spray parameters: 20 seconds spray pulse, 2.0 bar feed air pressure

**TABLE A2.2** Effect of electrostatically-charging the spray on the impact on surfaces of test objects

The impact of an electrostatic spray on the disc object was investigated in the spray chamber. Table A2.3 shows the mass of spray deposited on the surfaces of the disc object. The largest increase in deposition on the disc object due to electrostatic spraying was on the side. This was the surface that in normal circumstances, i.e. when the nozzle was uncharged, received the lowest impact in the spray chamber. The bottom surface was directly facing the spray nozzle and the top surface acquired spray because of the settling of spray droplets due to gravity. By charging the spray and earthing the object, a force
component acting towards the centre of the spray chamber would have been created, aiding the deposition of spray droplets on the side of the object. The increase in deposition on the top and bottom of the object are comparable with those shown in Table A2.2. The increase in impact on the side of the cylinder is larger than that previously observed.

The impact rate of an electrostatically-charged spray on the side of the disc object is shown in Figure A2.4. This figure contrasts with Figures A2.1 - A2.3 which show a constant impact rate on the surfaces of the test objects. At low exposure times, the impact rate on the sides of the cylinder was low and only small quantities of spray were deposited. Under these circumstances, the losses due to evaporation from the side of the cylinder prior to sampling would have been appreciable. Therefore the measured mass deposited on the side of the cylinder for short exposure times is likely to have been underestimated.

<table>
<thead>
<tr>
<th>SURFACE</th>
<th>MASS GAINED at 0 kV / mg cm²</th>
<th>MASS GAINED at -15 kV / mg cm²</th>
<th>RATIO -15 kV/0 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP</td>
<td>0.882</td>
<td>1.02</td>
<td>1.16</td>
</tr>
<tr>
<td>SIDE</td>
<td>0.053</td>
<td>0.311</td>
<td>5.86</td>
</tr>
<tr>
<td>BOTTOM</td>
<td>0.939</td>
<td>1.06</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Spray parameters: 1.5 bar feed air pressure, 20 second spray pulse

**TABLE A2.3** Comparison of impact of spray on disc object using uncharged and electrostatically-charged sprays

Figure A2.4 again demonstrates an increased deposition rate when the spray nozzle was raised to -15 kV potential. The ratio between the mass of spray deposited when the nozzle was charged compared to when the nozzle was uncharged decreased as more spray was deposited on the surface. As the mass of electrostatically-charged spray deposited onto the surface increased, so did the overall charge present, assuming that the object was not earthed. Repulsion between like charges will occur between the surface and the spray droplets, counteracting the force imposed on the spray droplets due to the electric field between the spray nozzle and the earthed bayonet socket fitting above the object. This will
FIGURE A2.4 Impact on the side of the disc object from electrostatic and non-electrostatic sprays
have the effect of lowering the impact rate as spray is accumulated on the surface. This explains in part why on the other surfaces of the squat cylinder where higher impact was observed, i.e. the top and bottom surfaces, electrostatic charging of the spray did not produce so marked an effect.

The effect on enhancing deposition of spray by attractive electrostatic forces can be limited by the momentum of the entrained droplets in the air streams surrounding the object. For example, the data in Table A2.1 showed that an increased impact could be obtained on the outside of the hollow stainless steel cylindrical object using electrostatically-charged sprays. When uncharged sprays were employed, no impact was obtained inside the cylinder when the bottom of cylinder was sealed. Even when electrostatically-charged sprays were employed, no impact was obtained inside the cylinder. As air could not flow through the cylinder (because it was sealed at the bottom) droplets would have to leave the surrounding air streams to enter the hollow cylinder. The absence of deposited spray inside the hollow cylinder could be explained by the entrainment of spray droplets in the surrounding air streams.

The forces produced by the momentum of the air streams created by the air atomising nozzle in the spray sterilisation chamber often would generally act to lessen the effect of the attractive forces of the electrostatically-charged drops to the target. In the absence of the bulk air movement in the chamber, the effect of the electric field was seen by the rapidly clearing electrostatically-charged mist (taking about 5 seconds to clear) compared to the uncharged case where the mist in the chamber took about 2-3 minutes to settle due to gravity.
APPENDIX 3. STATISTICS

A3.1 Introduction

This appendix contains four tables originally shown in Chapter 3 to which have been added the standard deviations of the experimentally-determined parameters for first-order and multi-target disinfection kinetics. The table numbers in this appendix are the same as in Chapter 3, e.g. Table 3.1 is Table A3.1.

All comparisons of inactivation constants were assessed using the Student t-test (Chatfield, 1970) and were all found to be significant at the 99% level.

A3.2 Results

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>INACTIVATION RATE CONSTANT (Jm⁻²)⁻¹</th>
<th>EXPONENT</th>
<th>LOG₁₀(EXPONENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Mean 1.65 x 10⁻² ± 9.88 x 10⁻⁴</td>
<td>Mean 2.65</td>
<td>Mean 0.423 ± 0.156</td>
</tr>
<tr>
<td>Wet Grade 2 filter paper</td>
<td>Mean 3.22 x 10⁻² ± 1.29 x 10⁻⁴</td>
<td>Mean 2.00</td>
<td>Mean 0.301 ± 5.09 x 10⁻²</td>
</tr>
<tr>
<td>Wet Grade 6 filter paper</td>
<td>Mean 9.34 x 10⁻¹ ± 7.11 x 10⁻⁴</td>
<td>Mean 2.00</td>
<td>Mean 0.301 ± 0.127</td>
</tr>
</tbody>
</table>

TABLE A3.1 Multi-target kinetic constants for B. subtilis spore inactivation by UV
INACTIVATION RATE CONSTANT (Jm⁻²)⁻¹

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass microfibre filter paper</td>
<td>1.10 x 10⁻³</td>
<td>1.78 x 10⁻⁴</td>
</tr>
<tr>
<td>Impervious materials (PTFE, aluminium and stainless steel)</td>
<td>2.28 x 10⁻²</td>
<td>1.74 x 10⁻³</td>
</tr>
<tr>
<td>Anodisc membranes</td>
<td>2.13 x 10⁻²</td>
<td>2.09 x 10⁻³</td>
</tr>
<tr>
<td>Dry Grade 2 filter paper</td>
<td>4.79 x 10⁻³</td>
<td>1.43 x 10⁻⁴</td>
</tr>
<tr>
<td>Dry Grade 6 filter paper</td>
<td>8.77 x 10⁻⁴</td>
<td>1.34 x 10⁻³</td>
</tr>
</tbody>
</table>

TABLE A3.2 First-order kinetic constants for *B. subtilis* spore inactivation by UV

<table>
<thead>
<tr>
<th>Species</th>
<th>Inactivation rate constant</th>
<th>Target number</th>
<th>LOG₁₀ (Target number)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em> (ATCC 6633)</td>
<td>3.81 x 10⁻²</td>
<td>9.47 x 10⁻³</td>
<td>172</td>
<td>This work</td>
</tr>
</tbody>
</table>

TABLE A3.4 Summary of multi-target parameters for *Bacillus* spores exposed to 25.8% or 29% H₂O₂

<table>
<thead>
<tr>
<th>UV + 1.0 w/v% H₂O₂ first-order kinetics</th>
<th>Inactivation rate coefficient (Jm⁻²)⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Mean</td>
</tr>
<tr>
<td>Wet Grade 2 filter paper</td>
<td>1.02 x 10⁻¹</td>
</tr>
<tr>
<td>Wet Grade 6 filter paper</td>
<td>4.95 x 10⁻²</td>
</tr>
<tr>
<td>Aluminium coupons</td>
<td>1.29 x 10⁻¹</td>
</tr>
</tbody>
</table>

TABLE A3.6 Comparison of kinetic parameters for the UV and UV + 1.0 % H₂O₂ process on different surfaces and in distilled water

230
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