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THE CHARACTERISATION OF A THIN FILM UV CONTACTOR AND ITS APPLICATION TO THE TREATMENT OF CONTAMINATED CUTTING OILS

by

Christopher James Peppiatt

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of

Doctor of Philosophy of Loughborough University

June 1997

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ABSTRACT

The characteristics and applications of a novel design of a thin film photocatalytic contactor based on the principle of irradiating a 'water bell' with ultraviolet (UV) light are considered in this work. Measurements of UV doses received by the liquid films in single passes were made using both actinometric and bioassay-based methods. The chemical actinometer employed was potassium ferrioxalate (K₃Fe(C₂O₄)₃) and the microorganisms used in the bioassay were Pseudomonas stutzeri (IBRG) and a repair-deficient strain of Escherichia coli (NCIMB 11190). Good agreement was obtained between the doses measured using actinometry and the E. coli-based bioassay. At higher doses, good agreement was also obtained for the dose estimates made using actinometry and the Ps. stutzeri bioassay. In addition, a hydrodynamic water bell model, previously developed in the literature, was combined with a UV intensity model to predict UV doses with generally good results.

Microbially contaminated metal working fluids were identified as a suitable medium for disinfection using the thin film contactor because they are not treatable using conventional UV contactors, and because the systems employed in industry vary widely in scale. Batches of contaminated emulsion ranging in volume from 200 to 1000 L were successfully disinfected. Representative members of the microbial population were isolated, and their changing status throughout treatment recorded. Against expectations, the population showed no capacity for the post-irradiation repair of UV-induced damage.

A simplified disinfection model was established in order to model the treatment of batches of contaminated metal working fluids. Preliminary predictions made using a combination of experimental data for the population as a whole and that for individual species coupled with that generated using the hydrodynamic bell model, gave encouraging results.
ACKNOWLEDGEMENTS

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Finally I would like to thank Mrs. Pietertje Cooper for allowing me to stay on at her house while completing this thesis.
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CHAPTER 1: INTRODUCTION

The use of ultraviolet (UV) light for the disinfection of liquids is relatively well-known. It is widely used in the treatment of water: e.g. secondary sewage works effluent (Nieuwstad et al., 1991), drinking water (Wolfe, 1990; Lauer et al., 1991), sewer overflows (Boner et al., 1995), sea water for use in offshore drilling (Brunner and Klein, 1985), in swimming baths, and for the disinfection of aquacultural water (Liltved and Landfald, 1996). UV disinfection has also been proposed for the treatment of blood products (Benesi, 1956; Oppenheimer et al., 1959), dialysis fluid (Tolon et al., 1977), and foodstuffs such as cider (Harrington and Hills, 1968), milk (Curran and Tamsma, 1960) and sugar syrups (Murphy, 1965; Kissinger and Willits, 1966a, 1966b). The UV irradiation of liquids such as fruit juices, beers, and wines has not been commercially viable due to limitations (i.e. suspended solid particles or chemical instability) imposed by these products (Shechmeister, 1983).

Lambert's law states that the proportion of radiation absorbed by a non-diffusing absorbing medium is independent of the incident intensity (i.e. each succeeding element of the absorbing medium of thickness dx absorbs an equal fraction of the radiation of intensity I which is incident upon it). This may be expressed as:

\[ I = I_o \cdot e^{-bl} \]  \hspace{1cm} (1-1)

(where \( I_o \) = the radiant intensity before passage through the medium, \( b \) = the absorption coefficient of the medium, \( l \) = the thickness of the medium, and \( I \) = the radiant intensity at depth \( l \)).

Clearly, if the depth of liquid is too great, or the absorption coefficient too large, UV disinfection becomes inefficient (Shama 1992a). The absorption coefficient may be purely a measure of the UV transmittance characteristics of
the liquid itself, but it may also be affected by e.g. the presence of suspended particles in the liquid (Qualls et al., 1983). The consequence of excessive UV attenuation may be that the flow rate of liquid undergoing treatment has to be reduced to decrease the probability of significant numbers of live cells being present in the outflow. Variations in the UV transmittance of a treated liquid may cause the failure of an installation to meet a standard minimum requirement for treatment (Tobin et al., 1983). The fouling of UV light sources (which may be caused by oil and/or grease in the treated liquid, adherent solids from the liquid, biofilm growth, calcium or iron precipitates, or by hardness-scaling) is another problem associated with the UV disinfection of liquids. Fouling causes a reduction in the intensity of UV light reaching the treated liquid, thereby limiting the effectiveness of the process (Kreft et al., 1986).

As a solution to these problems, it has been suggested that liquids be irradiated as films or sheets (Benesi, 1956; Oppenheimer et al., 1959; Curran and Tamsma, 1960; Ashton et al., 1988; Shama, 1992a, 1992b). Shama (1992a) described an experimental apparatus in which liquids were treated in the form of 'water bells'. These are totally enclosed thin liquid sheets which are hollow and, as their name suggests, bell-shaped. The hollow nature of liquid bells makes it possible to avoid permanent contact between the treated liquid and the sleeves (usually quartz or PTFE) of the light sources. In this way, fouling of the sources, either by the treated liquid, or by substances present in it, can be reduced.

1.1: OBJECTIVES OF THE WORK

It was proposed that the project be to characterise the performance and operation of UV contactors which utilise the liquid bell principle. The tasks involved were to comprise:
(1) Evaluating the performance of a thin film UV contactor in disinfecting contaminated metal working fluids (MWF). It was envisaged that the system would be operated in such a way as to treat batches of fluid by recycle.

(2) Measurement of the UV doses delivered to the liquid bells by various combinations of UV sources. Both actinometric and bioassay-based methods were to be used and compared.

(3) The evaluation of existing hydrodynamic models for liquid bells and the coupling of such models with a model enabling UV intensities to be estimated over the entire surfaces of liquid bells. The objective of this was to enable the doses delivered to the bells by various arrangements of UV sources to be estimated from the combined model and verified using the experimentally-determined data.

(4) The establishment of a simplified model to enable inactivation kinetics in a batch system undergoing irradiation to be predicted. This model was to be applied to the disinfection of contaminated MWF and comparisons were to be made with experimental data.
CHAPTER 2: LITERATURE SURVEY

2.1: DISINFECTION WITH UV LIGHT

The ultraviolet region of the electromagnetic spectrum is sub-divided into three regions: UVA ('near UV') from 400 to 315 nm, UVB from 315 to 280 nm, and UVC ('far UV') from 280 to 200 nm. The UV region below 200 nm ('vacuum UV') is strongly absorbed by air.

The antimicrobial effects of sunlight have been known since the 19th century (Downes and Blunt, 1877). Early research indicated that this effect was due to the UV portion of sunlight. The optimum wavelength range for disinfection is from 250 to 266 nm (Luckiesh, 1946), indeed, far UV is of the order of a thousand times more effective in killing microbes than near UV (Jagger, 1967). An action spectrum displays the relative efficiencies of incident photons, as a function of their energies, in producing a given biological effect (Harm, 1980). As long as the quantum yield (i.e. the number of moles of photoproduct produced per mole of photons) for the relevant photoproduct(s) is approximately constant over the spectral region examined, the action spectrum will resemble the absorption spectrum of the molecules responsible for their formation. Although dose sensitivity varies from species to species, and from strain to strain within species, action spectra for UV disinfection always peak in the aforementioned region. Nucleic acids (DNA and RNA) constitute the genetic information of any cell, and damage to even a single gene may cause the mutation or death of that cell. By comparison, the loss of a few molecules of any other type of compound would be unlikely to have significant consequences. It follows, therefore, that nucleic acids are the most sensitive molecular species in microbes. Nucleic acids show an absorption peak around 260 to 265 nm, which correlates well with peak UV microbial inactivation (Harm, 1980). This correlation between peak inactivation wavelength and nucleic acid peak UV absorption, coupled with the biological
importance of nucleic acids, provides strong evidence that absorption of photons by nucleic acids is responsible for the majority of killing and mutation effects caused by far UV.

UV technology became established in the early 20th century with the development of the mercury vapour lamp by Hewitt in 1901 (Koller, 1965). It is highly fortuitous that the peak emission of the low pressure mercury arc lamp is at 253.7 nm, a wavelength that is 85% as effective for microbial killing as the theoretical optimum at 265 nm (Meulemans, 1987). Since a typical lamp will convert 35%-60% of input power into photon emission at 253.7 nm (Meulemans, 1987; Phillips, 1983), and is relatively cheap, such lamps are widely used for UV disinfection.

2.1.1: The Effect of Temperature on UV Disinfection

Photochemical reactions are insensitive to changes in the temperature of reacting species (Calvert and Pitts, 1966). This insensitivity has been suggested as a major advantage of UV disinfection over the use of chemical disinfectants (e.g. chlorine or ozone), whose inactivation kinetics are sensitive to temperature change (Severin et al., 1983b). This assumption would be true if UV disinfection were a simple photochemical reaction. The physiological act of inactivation is not completely understood, and the existence of cellular repair mechanisms for UV damage further complicates the situation. Whilst the accumulation of UV damage is a purely photochemical reaction, all of the reactions which are involved ultimately in the process of inactivation may not be so. Thus it is possible that, during any period of cellular exposure to UV light, alterations in temperature may have effects other than on the original photochemical event. A possible example of this could be the temperature-induced modification of repair enzyme kinetics, or the effect of temperature on the regrowth of cultures treated by circulation through a UV contactor (see 7.4.1).
Severin et al. (1983b) conducted experiments on the effects of temperature on UV disinfection, using a bacterium (Escherichia coli), a yeast (Candida parapsilosis), and a virus (f2) irradiated at temperatures of 5 °C, 20 °C and 35 °C. It was found that, for E. coli and C. parapsilosis, the inactivation rate constant, k, was approximately 10% greater at 35 °C than at 5 °C; for the f2 virus the increase was approximately 17%. UV inactivation energies for the three organisms were found to be very low (<1,050 cal/g.mol); a figure almost an order of magnitude less than those observed for some chemical disinfectants. However, Abu-ghararah (1994) investigated the kinetics of wastewater disinfection and found that there was no statistically significant effect due to temperature variation in the range of 20-40 °C. It seems, therefore, that liquid temperature is not a major factor as far as the initial inactivation of microorganisms by UV is concerned, within the temperature ranges studied (5-40 °C).

A further possible effect of temperature on the efficiency of UV disinfection is the effect of lamp temperature on spectral output. The highest efficiency occurs at 40-41 °C, while lamps operating at temperatures of either 27 °C or 66°C are only about 50% as efficient. This effect is due to the change in mercury vapour pressure caused by changing tube lamp temperature, the optimum pressure for maximum lamp efficiency being reached at the above tube temperature (Phillips, 1983).

2.1.2: The Rôle of Suspended Particles in UV Disinfection

Suspended particles in a liquid which is to be treated with UV light may affect the process in two ways:

(1) Particles can shade cells from UV light (as is also the case where microorganisms form clumps). Furthermore, cells may become attached to
individual particles, or be sheltered within particle aggregates. Oliver and Cosgrove (1975) found that samples were more sensitive to UV treatment if they had been previously dispersed by ultrasonication.

(2) Particles may either absorb, or scatter UV light. Thus humic material is a strong absorber of UV light, whereas materials such as clays and micas cause strong scattering effects. Spectrophotometric measurements overestimate UV absorbance in liquids where there is scattering caused by suspended particles: it has been found (Qualls et al., 1983) that scattering accounted in one case for 12% of the spectrophotometric absorbance of the sampled liquid.

Survival curves for systems where shading/shielding effects occur are typically sigmoidal with an initial shoulder, a linear portion, and a final plateau of resistance at higher dose values. The final plateau is, obviously, due to the survival of microorganisms, either within clumps, or associated with suspended particles.

2.1.3: Mixing Effects in UV Disinfection

In practical UV disinfection it may be said that: inactivation kinetics are complex, photoinactivation is rapid, and UV intensity is not uniform (Severin et al., 1984c; Severin and Suidan, 1985). It may be concluded from this that UV disinfection is likely to be influenced by mixing. It has been observed that differing reactor designs do not give comparable inactivation efficiencies for comparable doses. Cortelyou et al. (1954) observed that increased turbulence in a flow-through reactor led to increased inactivation efficiency, suggesting that the effect might be due to improved transport of microorganisms to the vicinity of the lamp.

The work of Severin et al. (1984c) showed that inactivation increased when a completely mixed flow-through reactor took on elements of plug flow
behaviour (i.e. mixing became incomplete). It was suggested that radial mixing (i.e. perpendicular to the direction of flow) is beneficial for UV inactivation, whilst longitudinal mixing (i.e. in the direction of flow) is damaging to reactor efficiency. The conclusion reached was that it is probably useful to allow some longitudinal mixing in order to avoid the occurrence of radial stratification. A hydraulic study of commercially-used UV disinfection units (Kreft et al., 1986) indicated that reactor designs in which there are near plug flow conditions were more energy-efficient than those in which there was significant liquid short circuiting.

As has been stated (Severin et al., 1984c), the closer flow conditions approach ideal plug flow characteristics (i.e. longitudinal dispersion, $E_v = 0$), the greater the UV disinfection efficiency. Plug flow in UV reactors ensures minimal short circuiting and longitudinal dispersion in the treated liquid for turbulent flow conditions. In pipes and closed conduits, turbulent flow will occur at Reynolds Number ($Re$) values in excess of 2,000; it has been suggested (Thampi, 1990) that UV reactors should operate at $Re > 5,000$. Furthermore, if the length to hydraulic radius ratio ($L/R_h$) of a reactor is greater than 50, radial mixing is minimal (Thampi and Sorber, 1987).
2.2: THE BIOLOGICAL EFFECTS OF UV LIGHT

2.2.1: Photoinactivation of Living Organisms

Ultraviolet photons forming a uniform beam which strikes a thin component of a material of constant composition are absorbed in a random fashion, with no one molecule more likely to absorb a photon than another. A relatively large number of photons usually have to be absorbed by a cell before it is altered or inactivated. The actual inactivating event, however, is normally caused by only one of these photons. In this case, the rate of change (decrease) of the number of cells still active (i.e. capable of division, and thus forming a colony) with respect to applied dose is proportional to the number of active cells remaining at that dose level. Thus the rate of change of active cells with respect to dose can be expressed as:

\[ \frac{dN}{dD} = kN \]  

(2-1)

(where \( N \) = number of surviving particles at dose \( D \), and \( k \) = the inactivation constant).

Integrating this expression gives:

\[ \ln \frac{N}{N_0} = -kD \]  

(2-2)

or alternatively,

\[ \frac{N}{N_0} = e^{-kD} \]  

(2-3)

(where \( N_0 \) = the number of viable cells at time zero). This expression is known as the Chick-Watson Law (Chick, 1908; Watson, 1908).
The quantity \(\frac{N}{N_0}\) is termed the 'surviving fraction'. Inactivation data are often quoted as such, or in the form of percentage survival figures. Equation (2-3) can also be written as:

\[
\frac{N}{N_0} = e^{-kt}
\]

(2-4)

(where \(I = \text{intensity, and } t = \text{exposure time}\)).

The inactivation rate of a pure culture of microorganisms is first order with respect to local UV intensity, and first order with respect to the number of surviving organisms. In batch or plug-flow experiments with uniform radiation profiles, a semilog function of surviving fraction against the product of intensity and time (I.t) is regularly observed (Qualls and Johnson, 1983; Qualls et al., 1983; Severin et al., 1983a; Severin et al., 1984a; Severin and Suidan, 1985). Such a pattern is consistent with results for chemical disinfection methods (where exposure is equal to the product of sterilant concentration and exposure time, C.t), and the efficiencies of differing disinfectants are often compared using this 'C.t' evaluation. Results are usually quoted for 90, 99, or 99.9% cell inactivation. A given exposure (i.e. equal values of C.t, or I.t) should give the same inactivation value for a given strain of organism with a given disinfectant. This relationship is known as the Bunsen-Roscoe reciprocity law (Bunsen and Roscoe, 1862). For the UV treatment of both bacteria and the spores of moulds this law has been shown to apply over a thousand fold range of intensity (Luckiesh et al., 1949).

The difficulty with using an inactivation model based on equation (2-4) is that the expression is of no use for situations when flow-through reactors not displaying perfect plug flow are used. Qualls and Johnson (1983) used Bacillus subtilis spores with flow-through reactors. Slug injections of spores were passed through the reactors with the lights off in order to establish a retention
time distribution. This experiment was next repeated with UV lights lit to give spore inactivation against time distribution. The results of these flow-through tests were next compared to a standard batch inactivation curve so that the dose for each flow fraction could be obtained. The average UV intensity for each fraction was approximated by dividing the dose by the time elapsed since injection. Thus the results are effectively in the form:

\[
\frac{\ln N}{N_0} = -k\tau
\]

(2-5)

(where \(\tau\) = the theoretical retention time in the reactor). This method is limited to the analysis of reactors with uniform light profiles.

Severin et al. (1984a) used pure cultures of organisms in a single-lamp, flow-through, annular UV contactor exhibiting complete mixing. This contactor was modelled by the mixed second-order expression:

\[
\frac{N}{N_0} = \frac{1}{1 + mk_{\ell} \tau}
\]

(2-6)

(where \(m\) = a dimensionless function of reactor geometry and water quality, representing the ratio of the average light intensity in the contactor to the intensity at the surface of the quartz tube covering the lamp, \(I_o\)).

Kinetic constants calculated using a mixed, second-order approximation (the gradient of the linear portion of a semilog plot of \(N/N_0\) vs. \(\tau\) was measured) for batch in activations of the same microbial species differed from those obtained using equation (2-6) for the flow-through data, and could not be used to predict behaviour in the completely mixed flow-through reactor when a resistant initial shoulder was found in the batch curve. Indeed, the importance of shoulder effects was demonstrated by the apparent reversal of resistance between the flow-through contactor and batch experiments. It was
found that, for batch inactivation experiments, the bacterial f2 virus was more UV resistant than the yeast C. parapsilosis. In the flow-through contactor, however, the yeast was the more resistant, due to the resistant shoulder exhibited by C. parapsilosis (the f2 virus, having single-stranded RNA as its genetic material, does not exhibit any resistance to inactivation at low doses, and therefore has no resistant shoulder to its inactivation curve).

The same group (Severin et al., 1983a) modelled initial resistance to UV inactivation with two standard kinetic models, one using multtarget kinetics, and the other using series-event (multi-Poisson) kinetics. The advantage of such models over empirical rate models is that they are derived from fundamental assumptions about rate, and can be applied to a variety of flow conditions. When these models were applied to the completely-mixed, flow-through contactor, good predictions for inactivation performance were achieved using kinetic parameters obtained from batch inactivation data.

Of the two models the series-event model is thought to correspond more closely to actual UV inactivation mechanisms. In the series-event model a unit of damage is termed an ‘event’. These events occur in steps, with each step an integer function. The rate at which any organism passes from one event level to the next is first-order with respect to UV intensity, and is independent of the level which the organism occupies. An organism continues to accumulate damage as long as it is exposed to UV light. Further, there is a threshold at which, once it is exceeded, organisms are inactivated. The threshold level will be constant for any given set of conditions, although it may vary for any species depending on culturing conditions (both before and after irradiation). The series-event model may be expressed as:

\[
M_0 \xrightarrow{k_1} M_1 \xrightarrow{k_1} \ldots M_i \xrightarrow{k_1} \ldots \xrightarrow{k_1} M_{n-1} \xrightarrow{k_1} M_n \xrightarrow{k_1} \ldots \quad (2-7)
\]
(where \( k \) = the mixed, second-order reaction rate constant, \( I \) = the local point light intensity, \( M_i \) = an organism which has reached event level \( i \), and \( n \) = the threshold level for the organism). For a completely mixed, flat, thin-layer batch UV contactor the density of surviving organisms can be expressed as:

\[
\frac{N}{N_o} = e^{-iu} \sum_{i=0}^{\infty} \frac{(k\bar{I}i)!}{i!}
\]  

(2-8)

(\( \bar{I} \) = average UV intensity, \( i \) = event level, and \( n \) = the threshold number of damaged sites required for inactivation). The rate model for series-event kinetics applied to the completely mixed, flow-through contactor is:

\[
\frac{N}{N_o} = 1 - \left[1 + \left(\frac{1}{mk\bar{I}o\tau}\right)\right]^{-n}
\]  

(2-9)

(note that \( mI_o = \bar{I} \)).

The above approach has been extended to study the combination of cell clumping effects and cellular UV resistance. The term multitarget series-event kinetics has been used for this model (Severin et al., 1984b; Severin and Suidan, 1985). The rate expression for batch UV inactivation is given as:

\[
\frac{N}{N_o} = 1 - \left(1 - e^{-iu} \sum_{i=0}^{\infty} \frac{(k\bar{I}i)!}{i!}\right)^L
\]  

(2-10)

(where \( N/N_o \) = the surviving fraction of clumps of cells in a uniform suspension of clumps with \( L \) cells per clump, and \( n \) = the threshold number due to internal UV resistance).

This batch rate inactivation expression may be included in material balances describing differing reactor/contactor configurations in order that
inactivation may be predicted in full-scale UV systems. When $L = 1$, the expression becomes the series-event model, and when $n = 1$, it becomes the multitarget model (Severin et al., 1983a).

For industrial applications, inactivation kinetics are complicated by the heterogeneity of the microbial populations and by clumping effects.

2.2.2: Cellular Recovery and Repair Following UV Damage

It has already been stated that nucleic acids are the most UV-labile constituents of living cells. Given this, it is not surprising that all known mechanisms of molecular repair act on nucleic acids (DNA in particular). DNA repair mechanisms are considered to be fundamental to all organisms, in some cases the same repair processes apply to DNA damage caused by UV, ionising radiations, photodynamic action, and various organic and inorganic substances (many of which are carcinogenic). Further, DNA damage resulting from faulty replication, recombination etc. can also be repaired. Historically, however, DNA repair mechanisms were discovered as part of the study of UV radiation biology. The extent of repair is most impressive in the case of lethal UV photoproducts in DNA (usually pyrimidine dimers). Some mechanisms of repair are seemingly specialised to deal with this type of damage only. As UV light forms part of the spectrum of sunlight, and is thus a recurrent environmental factor for many organisms, the possession by an organism of an efficient repair system confers a survival advantage.

2.2.2.1: Types of Repair Mechanism

DNA repair processes may be of one of three types:

(1) Reversal of UV-induced alterations. This is the method of action of photoreactivation, where UV-induced cyclobutane pyrimidine dimers are
monomerized by the so called 'photoreactivation enzyme' in the presence of near UV or short-wave visible light. In this type of repair DNA damage is corrected in situ.

(2) Replacement of UV-damaged DNA nucleotides. Excision-resynthesis repair falls into this category. In this case a UV photoproduct (together with some adjacent nucleotides) is excised from the DNA molecule, and the correct sequence is resynthesized.

(3) Combination of the undamaged parts of replicating DNA molecules. Damage goes un repaired or is bypassed until undamaged DNA sequences are replicated and combined in such a way that a double-stranded DNA molecule, identical to the original before damage occurred, is formed. The double-stranded nature of the normal DNA molecule makes this type of repair possible, since either strand carries the information necessary for replication.

2.2.2.2: Photoreactivation

Photoreactivation has been defined as a "reduction in response to far UV irradiation of a biological system, resulting from a concomitant or post-treatment with non-ionising radiation" (Harm, 1980). Photoreactivation phenomena have been observed in virtually all living cells (Jagger, 1958). However, not all species within any taxonomic group are photoreactivable (Jagger, 1958). Several types of effect are now known which fit the above definition of photoreactivation; mostly these are due to photoenzymatic repair (also known as 'photorepair'). Other effects have been found to be the result of light-stimulated dark repair (also known as 'indirect photoreactivation'), or of the direct photochemical monomerization of pyrimidine dimers.
Pyrimidines (thymine, cytosine and uracil) are a class of nucleotide base which form part of the structure of nucleic acids. The dimerization of two adjacent pyrimidines within one DNA strand ('intrastrand' dimer formation) may be the result of relatively low far UV doses (Jagger, 1967). Further, there is considerable evidence (Setlow, 1964) to specifically implicate the thymine-thymine dimer as the principal photoreactable damage in DNA.

Fig. 2.1.: Formation of a Thymine-Thymine Cyclobutane Dimer

Photorepair involves the action of an enzyme known as 'photolyase', or the photoreactivating enzyme. This enzyme usually requires light energy from the near-UV or blue visible wavelength range (from approximately 310 to 480 nm) to be effective (Jagger, 1958; Harm, 1980). The discovery of a cellular factor in photorepair, besides light, came with the observation that the photoreactivation of a phage virus needs illumination of the infected host cell; neither the exposure to light of the UV-irradiated virus outside of its host, or the separate exposure of host cells and virus causes recovery (Dulbecco, 1950). A dependence on temperature in photoreactivation first indicated that this cellular factor was an enzyme (Rupert et al., 1958). Further work (Rupert, 1962a, 1962b) revealed the following reaction scheme:
\[
E + S \xrightleftharpoons[k_1]{k_2} ES \xrightarrow[hv]{k_3} E + P \quad (2-11)
\]

(where \(E\) = photolyase enzyme, \(S\) = the substrate (a photorepairable UV lesion), \(ES\) = the enzyme-substrate complex, \(P\) = the repaired product, and \(k_1, k_2, k_3\) = rate constants). The photorepairable lesions which constitute the substrate for photolyase are cyclobutane pyrimidine dimers in DNA.

The amount of photoenzymatic repair in a biological system may be expressed by an index called the 'fluence-reduction' (or 'dose-modification') factor (Kelner, 1949). This factor (< 1.0) represents the maximum extent to which the effect of the applied UV dose (or fluence) is reduced as a result of photorepair. The complementary value (1 minus the fluence-reduction factor) gives the fraction of dose which is rendered ineffective (this is also called the 'photoreactivable sector'). The photoreactivable sector can vary between a maximum of 0.90, in the in vitro Haemophilus influenzae DNA system (Rupert et al., 1958), and a minimum of 0.20, for the T4 phage in E. coli B (Jagger, 1958). No biological systems are known in which all lethal UV lesions are photorepaired (Harm, 1980). Thus it is possible to distinguish between photorepairable and non-photorepairable UV lesions. Since virtually all pyrimidine dimers can be monomerized under optimum conditions for photorepair (Harm, 1980), it seems that non-photorepairable lesions are not pyrimidine dimers.

This conclusion seems surprising considering pyrimidine dimers are much the commonest type of lethal UV photoproduct. It should be realised, however, that a large proportion of pyrimidine dimers are corrected not only by photorepair, but also by 'dark repair' processes. Dark repair is the term used for various processes which do not require exposure of damaged cells to light. When photorepair is studied in any biological system which is also subject to dark repair, the photorepair of only those UV lesions which are not
dark-repaired is evaluated, even though photorepair usually precedes dark repair (Harm, 1980). If a minority of UV lesions are neither photo- nor dark-repairable the experimentally measured photoreactivable sector will be less than the proportion of lesions which are actually photorepaired. This prediction is confirmed by observation: the photoreactivable sectors of dark repair deficient E. coli strains are generally greater, 0.80-0.85, than those of strains capable of dark repair, 0.50-0.70 (Harm, 1980).

2.2.2.3: Excision-Resynthesis Repair

The major mechanism of dark repair is known as 'excision-resynthesis' repair, or simply 'excision' repair. In plain terms, a damaged section of a DNA strand is removed and replaced with undamaged nucleotide bases to restore the DNA molecule to its original state. Fig. 2.2 is a general plan showing the types of step involved in excision repair.
The first step in the process is the cleavage of the phospho-diester backbone of the DNA strand near to a damaged sequence (e.g. a pyrimidine dimer); this reaction is catalysed by the UV endonuclease enzyme. There is more than one proposed path for the next steps in excision repair, which include the resynthesis of the original nucleotide sequence and the degradation of the damaged sequence. It is thought that in most organisms a so-called 'patch-and-cut' mechanism operates in which DNA polymerase I catalyses both the repair replication of the sequence, and the concurrent degradation of the original damaged sequence. In some mutant cell strains excision repair appears to progress in a way which is different from that seen in wild type cells. For these cases, the 'cut-and-patch' mechanism has been proposed; degradation is thought in this case to be catalysed by an exonuclease, whilst repair replication is brought about later by the action of a DNA polymerase.
other than DNA polymerase I. Finally, the DNA strand rejoins due to the action of polynucleotide ligase.

Although excision repair has been studied mainly in the *E. coli* bacterium, it appears to be of importance in all classes of living organism (Harm, 1980). Excision repair is less specific than photoenzymatic repair (which deals with pyrimidine dimers only). Besides pyrimidine dimers, differing types of DNA lesion (e.g. other lesions caused by photochemical action, chemical action, ionising radiation, and dye-sensitised photodynamic action) can be excised. Excision repair can be considered as a generalised DNA repair system, whose function is the recognition and removal of various kinds of damage (Harm, 1980).

2.2.2.4: Recombination (Postreplication) Repair

Recombination repair, like excision repair, is a dark repair process. Unlike excision repair, at least partial DNA replication must occur before recombination repair can operate (thus the alternative name 'postreplication repair'). Rupp and Howard-Flanders (1968) first obtained evidence for the mechanism of recombination repair. On studying DNA replication in excision repair deficient *E. coli*, they found that, following UV irradiation, newly synthesized nucleotide sequences were shorter than where there was no irradiation. However, it was found that, with the passage of time, this difference disappeared, as shorter nucleotide sequences were either converted (or integrated) into those of normal length. Fig. 2.3 shows the steps in recombination repair in *E. coli*. 
Fig. 2.3: Plan of Reaction Steps in the Recombination Repair of *E. coli*
(From W.D. Rupp *et al.*, *J. Mol. Biol.*, 61, 25, 1971)

(A) UV-irradiated double-stranded DNA.
(B) The same DNA during replication, the daughter strands (shown as thin lines) have gaps where the parent strands contain dimers.
(C) The gaps are filled with DNA sequences from the homologous parent strands by means of recombination.

There is a close relationship between recombination repair and genetic recombination; many of the genes involved in genetic recombination in *E. coli* also affect its UV sensitivity (Harm, 1980).

2.2.2.5: Other Types of Repair Mechanism

It has been found that a repair process involving the function of the *recA* and *lexA* genes (both of which are involved in recombination repair) is inducible by far UV radiation itself (Walker, 1984). It has been shown that UV-irradiated *E. coli* cells synthesise a protein which is not observed in either unirradiated cells of the same strain, or in irradiated or unirradiated cells of *recA*⁻ or *lexA*⁻ strains (Sedgwick, 1975). This inducible pathway is often called 'SOS' repair (Harm, 1980). Several other types of UV repair are known (Harm, 1980), but they are not as important as those already mentioned. These mechanisms are
generally concerned with viruses, and involve either repair in viruses, or their repair by host cell systems.

2.2.2.6: Repair in UV-Treated Liquids

The majority of studies involving the UV disinfection of liquids have investigated the reduction of microbial populations immediately after treatment, and have neglected the possibility of cell reactivation at a later stage. Much of the available data refers primarily to photoreactivation in UV-treated wastewaters, although dark repair phenomena have been noted (Mechsner et al., 1991). Many workers have claimed that recovery effects are significant (Harris et al., 1987b; Mechsner et al., 1991; Schoenen and Kolch, 1992; Lindemauer and Darby, 1994; Chan and Killick, 1995), whilst others maintain that they are negligible (Whitby and Palmateer, 1993). Chan and Killick (1995) have demonstrated that salinity has a deleterious effect on the photoreactivation ability UV-treated bacterial cells. At salinity levels of 10 gL⁻¹ salts the degree of photoreactivation was found to decline sharply, reaching a value of 5% of maximum recovery at a salt concentration of 30 gL⁻¹.

2.2.3: Repair-Related Phenomena

2.2.3.1: Liquid Holding

The survival of UV-irradiated E. coli B can be greatly enhanced by holding the cells for periods of time in buffer solutions (Roberts and Aldous, 1949). This effect is known as 'liquid holding recovery', and it is found in yeasts as well as prokaryotes. It seems that, at least in E. coli, the effect is due to the modification of the extent of excision repair. The amount of liquid holding recovery in different excision repair proficient bacterial strains is variable, and the effect may be absent completely. In some cases there may even be a decrease in survival with time after liquid holding (Harm and Haefner, 1968).
Both of these effects in *E. coli* are inhibited by substances such as caffeine or acriflavin, which bind to DNA, and are known to inhibit excision repair. Since excision repair involves several different enzymes, maximum repair will depend on a balance of their reactions. For industrial applications of UV disinfection, the practical impact of liquid holding will depend on temperature, nutrient content of the treated liquid, and the residence time of the liquid before use (Liltved and Landfald, 1996).

2.2.3.2: Dose Fractionation and/or Protraction

The Bunsen-Roscoe reciprocity law (Bunsen and Roscoe, 1862) states that the extent and type of photoproduct formation depends only on the applied dose, i.e. on the product of intensity and time. This is true from the purely photochemical point of view, but the UV inactivation of cells is the result of both photochemical and biological processes. Protraction of UV exposure over a long period of time, or its fractionation into several segments (separated by intervals where there is no exposure) can, therefore, affect dose-survival kinetics in some situations (Harm, 1968). One possible reason for such an effect might be a change in the physiological state of the cells during their UV treatment; e.g. cells may have different UV sensitivities at different stages of the cell cycle (Harm, 1980).

Dose fractionation can cause a change in the distribution of photoproducts with time, this in turn, can affect the amount of repair (Harm, 1968). It has been shown that the fractionation of dose causes greater cell survival when compared to that observed for the same dose applied in a single burst (Harm, 1968). Since the survival of excision repair deficient cell strains appears to be unaffected by dose fractionation, it would seem that dose fractionation allows enhanced excision repair to occur (Harm, 1968). The important factor seems to be the rate of photoproduct formation in DNA, allowing repair of existing lesions concurrent with the formation of new ones (Harm, 1968). As a result of
dose fractionation, the repair system is at no time confronted with as many UV lesions as are present after a single unbroken irradiation. The consequence of this is that the ability of the repair system to cope with damage is not overrun by photoproduct formation. This effect is important when the treatment of a volume of infected liquid by recycle past UV sources is studied.

2.2.3.3: Photoprotection and Indirect Photoreactivation

The phenomenon of photoprotection is defined as increased survival following far UV irradiation, due to a preceding exposure to near UV or short wave visible light. This effect is much less common than enzymatic photoreactivation, and is not observed in all strains of those species in which it does occur (Harm, 1980). The action spectrum for photoprotection is narrower than for enzymatic photoreactivation in E. coli, with a maximum at 340 nm, declining as wavelength increases to 400 nm, above which there is no effect (Jagger, 1964; Harm, 1980).

An effect resembling photoprotection may also be seen after postillumination. The action spectrum is indistinguishable from that of photoprotection, and the effect has only been observed in a mutant of a photoprotectable strain (e.g. E. coli B phr'), since it is otherwise impossible to exclude an increased survival due to enzymatic photoreactivation (Jagger, 1964). The postillumination phenomenon fits the operational definition of photoreactivation (see 2.1.6.2), for which reason it has been termed 'indirect photoreactivation' to distinguish it from the more commonly encountered enzymatic photoreactivation mechanism. In E. coli the effect usually occurs in the logarithmic growth phase (Jagger and Stafford, 1965).

It appears that photoprotection and indirect photoreactivation are based on the same mechanism (Jagger, 1964). Since both effects occur only in excision repair proficient strains, it seems that an increase in the amount of excision
repair is involved. The manner in which excision repair is stimulated is not at present known. It has been suggested that there is an induced growth delay that allows excision repair of UV lesions to occur for a longer time before the defects are fixed by replication (Jagger, 1964). However, photoprotection in the yeast Candida guilliermondii is not attended by growth delay at all (Fraikin et al., 1981). It seems that, as well as causing growth delay in many cases, photoprotection and indirect photoreactivation induce the synthesis of compounds which protect the cell from the effects of UV damage (Fraikin and Rubin, 1979).

Fraikin et al. (1981) suggested that the photoactivated synthesis of serotonin could be involved in near UV-induced photoprotection in C. guilliermondii; they proposed that serotonin (which binds to DNA) could protect cellular DNA against lethal lesions by trapping excitation energy from DNA in its own structure. In an apparent contradiction, the same authors noted that treatment with near UV light can also potentiate far UV killing in the same organism- in this case it was postulated that serotonin produced following near UV irradiation can interfere with the action of repair enzymes when bound to DNA. A similar potentiation of far UV-induced damage following near UV treatment was noted by Tyrrell and Webb (1973).
2.3: UV DOSE ESTIMATION

Since the efficiency of UV inactivation depends on applied dose, it is necessary to estimate the latter in order to evaluate the former. For flow-through UV contactors, the hydraulic characteristics of the contactor/reactor will give rise to a distribution of residence times. Since dose is defined as intensity multiplied by time, it is necessary to calculate both the residence time and the light intensity profile. For some flow regimes, however, it can be demonstrated theoretically that the average intensity ($\bar{I}$) is all that is required to model dose (Severin and Suidan, 1985). This is the case for both perfect plug flow and complete mixing. Whenever flow is stratified perpendicularly to the direction of flow, however, it follows that some part of the flow will travel through areas of the reactor which are exposed to below average UV intensities. Such flow conditions will control the quality of the treated liquid. Methods of dose estimation which have been suggested include: bioassays, chemical actinometry, and mathematical modelling.

2.3.1: Bioassay Dose Estimation

A variety of workers (Sugawara et al., 1981; Qualls and Johnson, 1983; Qualls et al., 1983; Harris et al., 1987a; Qualls et al., 1989) have used continuous flow bioassay methods with success to estimate UV dose in photoreactors. The method used by Qualls and his co-workers (Qualls and Johnson, 1983; Qualls et al., 1983; see 2.2.1) calculates the effective dose for a flow-through UV reactor by comparison of counts after a plug injection of unirradiated cells with an irradiated plug injection of cells as a function of time. A standard batch inactivation curve generated by using a collimated UV source to irradiate a stirred suspension of bacterial spores was used. In cases where liquid absorbance was low, the measured incident UV intensity was used to calculate the batch dose. When liquid absorbance was significant, however, the average intensity was calculated by the integration of Beer's Law over the
depth of the liquid (Morowitz, 1950). Survival ratios for each time element were then compared with the batch inactivation curve to estimate dose and intensity. Quintern et al. (1992) have proposed a bioassay system based on the dried spores of *B. subtilis*, immobilised onto plastic sheets, for determining the UV dose in sunlight. The high resistance of *B. subtilis* spores to UV (Meulemans, 1987) might render this method useful in measuring the high UV intensities encountered in photoreactors.

These bioassay methods have been shown to work for an annular, flow-through reactor with a radius not much greater than the lamp radius, and using a liquid with low UV absorbance (Qualls and Johnson, 1983; Severin et al., 1984a; Qualls and Johnson, 1985; Harris et al., 1987a; Qualls et al., 1989). Such conditions favour an environment with uniform light intensity, where even flow stratification will not affect inactivation. Harris et al. (1987a) have pointed out that dose can be inferred from microbial survival figures for continuous flow reactors by comparison with dose-survival relationships from batch inactivation studies only when the reactor's hydraulics are perfectly plug flow. As reactor hydraulics approach complete mix, microbial survival rates would be expected to rise, and the dose will be underestimated.

2.3.2: Actinometric Dose Estimation

An actinometer is a compound which undergoes reaction when exposed to light. For a photochemical system where the quantum yield (i.e. moles of photoproduct generated per moles of photons) of the photoproduct has been determined over a range of wavelengths, dose and intensity can be calculated if the concentration of photoproduct produced and the wavelength of the light from an incident monochromatic light source are known.
2.3.2.1: Types of Chemical Actinometer

(1) The uranyl oxalate actinometer (Leighton and Forbes, 1930; Heidt and Daniels, 1932; Brackett and Forbes, 1933; Forbes and Heidt, 1934) depends on the uranyl ion-photosensitised decomposition of oxalic acid. Irradiation of dilute aqueous solutions of oxalic acid and a uranyl salt (usually oxalate or sulphate) causes the decomposition of oxalic acid thus:

\[ \text{H}_2\text{C}_2\text{O}_4 \rightarrow \text{H}_2\text{O} + \text{CO}_2 + \text{CO} \quad (2-12) \]

This equation is an oversimplification of the reaction which occurs: \( \text{U}^{2+} \) and \( \text{HCO}_2\text{H} \) are also major products, the product distribution being influenced by pH (Carter and Weiss, 1940). Oxalic acid decomposition occurs in the wavelength range 435-208 nm, and the quantum yield for the reaction varies across the spectral range. Quantum yield does not, however, show sensitivity to actinometer concentration or to the intensity of incident light, and only slightly so to variations in temperature. Oxalate loss may be determined by titration with an oxidant, usually potassium permanganate or ceric sulphate. Unfortunately, this actinometer exhibits low sensitivity at low light intensities, where lengthy periods of irradiation are required to avoid error. Various changes to the analytical method originally described have been proposed (Pitts et al., 1955; Porter and Volman, 1962) to improve the sensitivity of this system.

(2) Leucocyanide actinometers are based either on the crystal violet (Weyde and Frankenburger, 1931) or malachite green (Harris et al., 1935; Harris and Kaminsky, 1935; Calvert and Rechen, 1952; Fisher et al., 1967) dye varieties, with malachite green leucocyanide having become preferred. When an ethanolic solution of malachite green leucocyanide is irradiated it produces a green coloration (absorption peak 622 nm) which can be measured by spectrophotometry. The quantum efficiencies for the region 225-334 nm have
been measured, and are close to unity. The system is very sensitive, and able to measure low light intensities. Its major limitations are due to the absorption of UV light by the dye product (which acts as an internal UV filter), and the fading of the product in the dark.

(3) The best known and most widely used chemical actinometer in photochemical research is the potassium ferrioxalate system (Parker, 1953; Hatchard and Parker, 1956). When sulphuric acid solutions of K$_2$Fe(C$_2$O$_4$)$_3$ are irradiated, a simultaneous reduction of ferric (3+) iron to ferrous (2+) iron and oxidation of oxalate ions occurs. The reaction involved is:

\[
2\text{Fe}^{3+}(\text{C}_2\text{O}_4)^{3-} \xrightarrow{\text{hv}} 2\text{Fe}^{2+}(\text{C}_2\text{O}_4)^{2-} + 3(\text{C}_2\text{O}_4)^{2-} + 2\text{CO}_2 \quad (2-13)
\]

The actinometer is sensitive over a wide range of wavelengths (250-577 nm), absorbing a high percentage of incident light for a small path length between the wavelengths 253.7 and 400 nm. Practically all light is absorbed by a thickness of 1 cm of potassium ferrioxalate in this range (Calvert and Pitts, 1966). The photochemical product (Fe$^{2+}$) is developed with a complexing agent (1,10-phenanthroline), giving rise to the formation of a red complex with a very high absorption coefficient (510 nm). The spectrophotometric measurement of the amount of complex formed allows the amount of product to be calculated. Useful exposures may be of very short duration. Furthermore, the system gives highly reproducible results (Harris et al., 1987a), and the irradiated solution can be stored for long periods of time before analysis (Jagger, 1967).

Harris et al. (1987a) have adapted the standard batch test using potassium ferrioxalate (Parker, 1953; Hatchard and Parker, 1956; Calvert and Pitts, 1966; Schoenen et al., 1993) for use in a continuous flow-through test. It was found that reproducible dose estimates could be obtained using potassium ferrioxalate in UV reactors.
(4) Although the three liquid actinometer systems mentioned above are the most well-known, various other solution actinometers have been used including: Reinecke's salt, chloroacetic acid (photolysis at 253.7 nm), and other organic compounds. Of particular interest is the system reported by Nicole et al. (1990) in which photon flux at 253.7 nm is determined by the measurement of the decomposition of hydrogen peroxide.

2.3.3: Mathematical Models for Dose Estimation

Such models produce a dose distribution from an intensity field distribution, and a retention time distribution. Using kinetic parameters for relevant microbial species or communities (determined by means of batch studies) together with dose estimation information, it is possible to predict the efficiency of a given UV reactor. It must be remembered, however, that the hydraulic characteristics of a reactor must be directly measured. This means that any such model is not truly predictive since a prototype reactor must be built before the retention time distribution can be determined.

2.3.3.1: Light Intensity Modelling

A wide variety of models have been employed to estimate intensity fields (Alfano et al., 1986). The line source with parallel plane emission model (LSPP: Harris and Dranoff, 1965) assumes that the lamp is a line source in which each point emits light in parallel planes perpendicular to the lamp axis. The line source with spherical emission model (LSSE: Jacob and Dranoff, 1970) uses a point source summation in which each point source emits light in every direction and isotropically. Finally, the line source with diffuse emission model (LSDE: Akehata and Shirai, 1972) assumes that each point source emits light in all directions and in a diffuse manner. It seems (Alfano et al., 1986) that LSSE and LSDE models better approximate the nature of real lamp
emission, but a decision must be made as to whether the complexities involved in any type of model are justified in terms of the extent of improvement over a simpler model.

Extense source models take account of the three-dimensional nature of UV lamps, and thus the three-dimensional nature of light emission. There are two types of extense source model, those with volumetric emission (ESVE models), and those with superficial emission (ESSE models). ESVE models (Irazoqui et al., 1973), in which the source is considered to be made up of emitters uniformly distributed inside its volume, are more suitable for the analysis of germicidal UV lamps (which have no coating on the inside of the lamp glass) than ESSE models (Bandini et al., 1977; Stramigioli et al., 1977), in which the source is considered to be made up of emitters evenly distributed over its surface area.

Light intensity field models which have been applied to UV reactors have been of the line source type. Qualls and Johnson (1983; 1985) preferred to use a point source summation for a finite-length LSSE model, whilst Suidan and Severin (1986) compared an infinite line source model with radial emission to an LSSE model. They found that the latter predicted slightly higher average light intensities in some conditions.

When using point source summation (i.e. finite-length line source) models all calculations depend upon having an accurate measurement of point source output. Some workers have calculated the point source intensity using manufacturer's data, whilst others (Qualls and Johnson, 1983; 1985) measure lamp output by integration of the outputs measured in an arc around the lamp centroid in the plane of the lamp axis.
2.4: UV REACTORS

Current commercial UV contactors fall into two basic design types (Anon., 1986). UV lamps can either be submerged in the liquid to be treated (contact reactors), or suspended outside it.

In contact reactors, the submerged lamps are generally encased in UV-transparent quartz sleeves. These submerged quartz systems can be of a variety of configurations, and are generally described by the lamp arrangement relative to the flow direction, and by the hydraulic design of the reactor. Fig. 2.4 shows a design in which a lamp battery is encased in a sealed shell. Flow enters perpendicular to the lamps via the inlet, is redirected to flow parallel to the lamps, finally exiting the reactor via the outlet. A steel plate acts as a baffle, splitting the reactor cylinder in half down its long axis. The flow, therefore, is directed down one side of the unit, turning to flow up the other before discharge. This modification encourages plug flow by increasing the hydraulic radius ratio (see 2.1.3).

Fig. 2.4: Schematic of Quartz UV Unit in Vinton, Iowa (a) top view, (b) plan view (Anon., 1986)
Submerged quartz systems can also be arranged as open channel units, with gravity feed. Fig. 2.5 shows an example of the type.

Fig. 2.5: Example of open channel unit in Pella, Iowa (a) plan view, (b) side view of lamp unit only (Anon., 1986).

Commercial non-contact UV systems usually employ designs in which the liquid is carried in thin-walled UV-transparent PTFE pipes. The lamps are positioned outside (and parallel to) the piping.

There are two acknowledged problems with these commercial designs, attenuation of UV light by the treated liquid and fouling (see Chapter 1). In order to avoid the worse effects of both of these factors, it is necessary to employ a reactor design in which the liquid is treated as a thin film, and in which the liquid has little or no contact with surfaces through which UV light is required to pass. Oliver and co-workers (Oliver and Cosgrove, 1975; Oliver and Carey, 1976) suspended lamps over a weir in order to achieve a thin-film non-contacting system. More recently, Shama (1992a, b) has described another thin-film non-contacting system which employs the principle of the water bell.
2.4.1: Liquid Curtains and Water Bells

`Liquid curtain` is the name given to any type of unsupported thin liquid film. Liquid curtains make it possible to construct novel contactors for the UV irradiation of microbially contaminated liquids. Moreover, these contactors may be used in conjunction with advanced oxidation techniques (e.g. UV/ozone, UV/hydrogen peroxide, UV/ozone/hydrogen peroxide, UV/titanium dioxide).

Annular liquid curtains may be formed in two fashions:
1. by vertical annular nozzles (i.e. annular nozzles which discharge in the direction of gravity), or
2. by methods which cause liquid to be discharged horizontally, forming a free-flowing sheet. This sheet will ultimately be affected by gravity, taking the shape of a roughly cylindrical, hollow sheet.

The liquid sheets formed by method (2) above are known as ‘liquid bells’ or ‘water bells’. A liquid bell is “an axisymmetric thin sheet of water which moves freely in the surrounding air and has a bell-like shape” (Bark et al., 1979). This phenomenon was first investigated by Savart (1833). They are formed by the use of horizontal annular orifice nozzles (Hopwood, 1952; Lance and Perry, 1953; Shama, 1992a), by the collision of a plane liquid jet with a surface (Taylor, 1959; Dumbleton, 1969), or by the direct impingement of two vertical liquid jets (Savart, 1833; Bond, 1935). If the liquid velocity is great enough a hollow, bell-shaped, three-dimensional thin sheet of liquid will be generated. Bells with film thickness lower than 1 mm have been generated (Shama, 1992a).

Liquid curtains have been proposed as chemical reactors (Roidt and Shapiro, 1985), and as protection systems in inertial confinement laser fusion reactors (Ramos, 1988). Shama (1992a) has demonstrated that liquids could be
irradiated in the form of bells. He used an arrangement of UV light sources permitting the irradiation of the very thin liquid sheet from both sides.

2.4.1.1: The Modelling of Liquid Bells

The modelling of liquid bells dates back to the work of Boussinesq (1869a, b). Later, Bond (1935) was able to deduce the surface tension of water by measuring the dimensions of a liquid sheet produced by the impacting jet method: he considered only the case where the pressures on the two sides of the film were equal. The work of Hopwood (1952) described the unusual shapes which can be exhibited by liquid bells when there is a pressure difference between the inside and the outside of the bell. In this work, the bells were projected into a water trough so that they were effectively sealed units. These findings were enlarged upon by Lance and Perry (1953) who found that a liquid bell could be expanded if air was introduced into the inside of the bell (thus making the internal pressure greater than the external pressure). The same two workers used a numerical solution for the differential equation for the bell surface to obtain the profiles of liquid bells.

Hovingh (1977) projected the equations of Lance and Perry onto a fixed cylindrical co-ordinate system. Later workers (Hoffman et al., 1980; Ramos, 1988) have also adopted this approach.
2.5: THE BIODEGRADATION AND MICROFLORA OF CUTTING OILS

2.5.1: Cutting Oils

The term 'cutting oil' is a generic one for formulations of mineral oils with additives which are diluted in water to concentrations typically between 2.5 and 5% by volume (Bennett, 1974). The functions of the resultant oil/water emulsion are as a coolant, and a lubricant for the metal working industries. Cutting oils are also referred to as 'metal working fluids' (MWFs). Many engineering companies maintain one or more large central sumps of cutting fluids, the contents of which are circulated to individual machines, and sprayed onto the workpiece during machining (Bennett, 1974).

A typical cutting oil concentrate consists of: mineral oils [naphthenic (cycloparaffinic) petroleum oils] 50-80%, petroleum sulphonates up to 10%, and fatty acids (oleic and linoleic acids from tall oils) less than 5% (Foxall-Van Aken et al., 1986). Other added ingredients include: corrosion inhibitors, coupling agents (to aid emulsion formation with water), antifoaming agents, lubricating agents and biocides (Saunders, 1990). The last category are present to prevent microbial proliferation in the diluted oil. When added to water, cutting oil concentrates form emulsions which appear either cloudy or milky.

2.5.2: Microbial Degradation of Cutting Oils

A major problem encountered by the users of cutting oil is the growth of microorganisms in the product. Symptoms of microbial spoilage in cutting oils may include the development of odours, a decrease in pH, changes in the stability of the emulsion, increased corrosion rates, surface-finish blemishes on workpieces, clogging of the coolant system (e.g. filters and lines), increased workpiece rejection rates, decreased tool life, unpredictable changes in coolant chemistry, and the increased incidence of dermatitis and other skin
conditions in operators (Passman, 1988). When water-based cutting oils become contaminated bacterial counts may exceed $10^9$ CFU/ ml (Foxall-Van Aken et al., 1986). It is thought that the primary carbon sources for microbial growth in cutting oils are the base mineral oil, the fatty acids, and the petroleum sulphonates. Little has been published on possible nitrogen sources in cutting oils, but nitrite from sodium nitrite (once extensively used as a corrosion inhibitor) has been implicated (Hernandez et al., 1985). Nitrates may also be present in the water used to dilute the oil concentrate (Bennett, 1973, 1974).

Bennett (1973, 1974) has written extensively on the factors which affect the biodeterioration of cutting oils. He considered three such factors:

(a) The presence of inorganic salts in the diluting water can greatly influence oil biodeterioration. In all cases, the amount of biodeterioration increases with increasing salt concentration. However, low levels of microbial growth are noted when cutting oils are diluted with distilled water- this indicates that there are enough essential ions present in some cutting oil concentrates to support microbial growth. It has been found that a mixture of inorganic salts, as is present in hard water, has a greater effect on biodeterioration than any individual salt. Chelating agents, present in some cutting oil concentrates for the purpose of emulsion stabilisation, also act to make ions unavailable, thus limiting microbial growth. Unfortunately, their presence causes foam production (Bennett, 1973).

(b) The ratio of oil concentrate to water is also important in the biodeterioration of MWFs. Cutting oils may be diluted in the range 1:1000 to 1:5 (0.1 to 20% by volume), but more usually from 1:40 to 1:20 (Bennett, 1974). The latter range is equivalent to that which is ideal for microbial growth (Bennett, 1974). Using the oil at higher concentrations does have an inhibitory effect on the growth of microorganisms, but such concentrations can cause the
formation of ‘invert emulsions’ which will not act in the desired manner (Bennett, 1973). Alternatively, using oils at concentrations less than 2% will result in fewer bacteria growing in the emulsion, possibly due to the lack of oxidisable substrate present in the environment (Bennett, 1974). Over dilution will lead to an emulsion which is unstable, and which will cause rusting in use (Bennett, 1973). Furthermore, increasing the dilution may mean that any biocide added to the original concentrate will be present at a concentration which is too low to have any effect on microbial growth (Bennett, 1974).

(c) Metal fines which are present in used cutting oils have varying effects on their spoilage flora. It is known that the presence of iron fines increases the oxidation of hydrocarbons (Zuidema, 1946), and will stimulate the biodegradation of soluble oils (Bennett, 1973, 1974). Other metal species have been variously claimed to have both antagonistic and synergistic effects on the action of different kinds of biocidal oil additives (Bennett, 1973; Law and Lashen, 1989; Rossmore, 1990; Sondossi et al., 1990).

The flora of contaminated cutting oils constitutes a diverse collection of microorganisms (see Table 2.1). The majority of bacteria isolated from cutting oils are Gram negative with *Pseudomonas spp.* featuring prominently. The ability of pseudomonads to utilise light oils is virtually a defining characteristic of the genus (King and McKenzie, 1977).
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>GRAM TYPE</th>
<th>REFERENCE(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter spp.</td>
<td>- ve</td>
<td>Salmeen et al., 1987</td>
</tr>
<tr>
<td>Aerococcus viridans</td>
<td>+ ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Aeromonas spp.</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>+ ve</td>
<td>Mattsby-Baltzer et al., 1989; Sandin et al., 1991</td>
</tr>
<tr>
<td>Citrobacter diversus</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>C. freundii</td>
<td>- ve</td>
<td>Bennett, 1974</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>+ ve</td>
<td>Mattsby-Baltzer et al., 1989; Sandin et al., 1991</td>
</tr>
<tr>
<td>Desulphobacterium spp.</td>
<td>- ve</td>
<td>Bennett, 1974</td>
</tr>
<tr>
<td>Diplodoccus spp.</td>
<td>- ve</td>
<td>Bennett, 1974</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>- ve</td>
<td>Passman, 1988</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>- ve</td>
<td>Bennett, 1974; Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>- ve</td>
<td>Bennett, 1974</td>
</tr>
<tr>
<td>Legionella feeli</td>
<td>- ve</td>
<td>Rossmore, 1993</td>
</tr>
<tr>
<td>Micrococcus spp.</td>
<td>+ ve</td>
<td>Bennett, 1974</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>- ve</td>
<td>Bennett, 1974; Passman, 1988</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>- ve</td>
<td>Sondossi et al., 1985b; Passman, 1988</td>
</tr>
<tr>
<td>Ps. alcaligenes (= Ps. pseudoalcaligenes = Ps. oleovorans)</td>
<td>+ ve</td>
<td>Rossmore and Rossmore, 1980; Mattsby-Baltzer et al., 1989; Sandin et al., 1991</td>
</tr>
<tr>
<td>Ps. stutzeri</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>- ve</td>
<td>Bennett, 1974</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Shewanella putrefaciens (= Ps. rubescens = Ps. putrefaciens = Alteromonas putrefaciens)</td>
<td>- ve</td>
<td>Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+ ve</td>
<td>Bennett, 1974; Mattsby-Baltzer et al., 1989</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>+ ve</td>
<td>Bennett, 1974; Mattsby-Baltzer et al., 1989</td>
</tr>
</tbody>
</table>

A variety of fungi have also been found growing in cutting oils, these include: Aspergillus spp., Candida spp., Cephalosporium (Acremonium) spp., Fusarium spp., and Trichoderma spp (Bennett, 1974; Passman, 1988; Sandin et al., 1991).

Apart from causing damage to cutting oil emulsions, microbial spoilage exposes operators to health risks (Fabian and Pivnick, 1953; Rossmore, 1993). Pathogens such as Salmonella spp. and Klebsiella pneumoniae are well-known from cutting oil cultures (Pivnick et al., 1954; Bennett and Wheeler, 1954). However, infections in metalworking operators from such pathogens have been only rarely reported. One such case was an outbreak of Pontiac fever among workers at an engineering firm at Windsor, Canada in 1981. The causative agent was Legionella feeli, the disease being a short-term, non-fatal legionellosis (Rossmore, 1993). The major threat to health caused by spoiled cutting fluids is to the skin. Both Staphylococcus and Streptococcus spp. have been implicated in 'metalworker's dermatitis', although it has been claimed...
(Bennett, 1974) that these bacteria generally occur as secondary infections and that the coolant, or preservative compounds in it, were the cause of the original skin problem (see below). It has also been suggested (Mattsby-Baltzer et al., 1989; Rossmoore, 1993) that airborne bacteria from cutting oils are a source of endotoxins. Endotoxins are lipopolysaccharide molecules present in the cell walls of Gram negative bacteria. Endotoxins from contaminated cutting oils may be responsible for, or at least exacerbate, the acute respiratory syndrome which is common among metalworkers.

2.5.3: Control of Microorganisms in Cutting Oils

2.5.3.1: Biocides Used in Cutting Oils

The use of biocidal additives as preservatives for cutting oils is the traditional method of controlling microbial growth. Biocides may be part of the formulation of the concentrate, or they may be added during use. Over the years biocides have been found to be a relatively efficient answer to the problem of biodeterioration in cutting fluids, but their use has certain disadvantages. Hernandez et al. (1985) listed skin irritation in workers, corrosion problems, high toxicity, and added handling and disposal costs as the drawbacks of biocide use. Furthermore it is very important to maintain an effective concentration of biocide in the bulk liquid, as low concentrations will be ineffective. It has even been reported that for some biocides there is a concentration range between bacteriostasis and ineffectuality at which microbial growth is stimulated (Bennett, 1974).

There are several types of compound which are either added specifically as biocides, or which have been attributed with biocidal effects in addition to their main function in the formulation. Phenols were used in oils as coupling agents, and also had useful biocidal properties (Saunders, 1990; Rossmoore, 1995). However, phenolic compounds have been almost eliminated from the
U.K. soluble oil market because of their high toxicity and consequent environmental risk (Saunders, 1990). Both alkanolamines (which are primarily corrosion inhibitors), and amine borates (which are emulsion stabilisers formed from alkanolamines and boric acid) have also been shown to have antimicrobial effects (Bennett, 1979; Hernandez et al., 1985; Sandin et al., 1991; Rossmoore, 1993). European water authorities are very concerned about the levels of boron-containing compounds in industrial fluids, and seem set to introduce strict controls on the levels of boron in effluent discharges (Saunders, 1990). The commonest groups of biocides used are the so-called ‘formaldehyde condensate adducts’ and the isothiazolones (Rossmoore, 1990).

Formaldehyde condensate adducts like 'Grotan BK' \([N,N',N''-\text{tris}-(\beta\text{-hydroxyethyl})\text{-hexahydrotriazine}]\) release formaldehyde into the cutting oil. Formaldehyde, due to its electrophilic nature, reacts strongly with nucleophiles (Sondossi et al., 1990). It is thought that the biocidal effects of these products is due to the reaction of cell nucleophiles (e.g. amino acids and lipopolysaccharides) with formaldehyde (Rossmoore, 1995). Formaldehyde-producing biocides are effective against many bacteria, but less so against fungi (Law and Lashen, 1989; Sondossi et al., 1990). Continued use of Grotan BK or similar products often results in a reduction in antimicrobial effectiveness. It has been proposed (Sondossi et al., 1990) that the reaction of formaldehyde with some nucleophiles in the system results in the loss of formaldehyde molecules. There have been suggestions (Sondossi et al., 1985a; Ohkawa, 1990) that one such nucleophile is the formaldehyde dehydrogenase enzyme which is responsible for formaldehyde resistance in microbes. 'Kathlon 886 MW' (methylchloroisothiazolone) is a widely-used example of the isothiazolone group of biocides. These compounds form the most recently-developed group of biocides, and are active at very low concentrations (Sondossi et al., 1990). The mechanism of action of isothiazolones is thought to be similar to that of formaldehyde, but isothiazolones are even more sensitive to some nucleophiles (Rossmoore,
Consequently, isothiazolones are similarly prone to the problems of loss of biocidal effectiveness seen with formaldehyde condensate adducts. A synergistic effect of Cu$^{2+}$ ions with both formaldehyde adducts and isothiazolones has been noted by several authors (Law and Lashen, 1989; Rossmoore, 1990; Sondossi et al., 1990). It is believed that copper ions compete with the biocides for certain nucleophiles which cause the deactivation of the biocide, thus extending its useful life. There is much debate in the metalworking lubricant industry as to whether it is advisable to abandon the use of biocides, and adopt some physical means of microbial control because of the extra cost of disposal of biocide-containing oil wastes, and possible future legislation against the use of biocides (Anon., 1993).

2.5.3.2: Physical Methods of Disinfection for Cutting Oils

In the light of the disadvantages associated with the use of biocides, several physical disinfection methods are being investigated. Pasteurisation has been evaluated with varying success (Hill and Genner, 1981; Rossmoore, 1995). Lewis (1987) proposed to use both pasteurisation and irradiation (either ionising or non-ionising radiation).

The application of advanced oxidation processes is unlikely to be suitable for the treatment of cutting fluids. It has been reported (Venkatadri and Peters, 1993) that Fenton’s reagent can degrade oils, and it is not difficult to see that the strong oxidants produced in advanced oxidation might attack the emulsion components as well as its microbial flora.
CHAPTER 3: MATERIALS, METHODS AND APPARATUS

3.1: BIOLOGICAL MATERIAL AND CULTURE MEDIA

3.1.1: Microbial Strains

The bacterial strains used for bioassay work were *Pseudomonas stutzeri* (IBRG), and a repair deficient strain of *Escherichia coli*, (NCIMB 11190). This particular *E. coli* strain has two gene mutations, *uvrA* (in consequence of which the bacterium does not possess the capability of excision-resynthesis repair, see 2.2.2.3) and *recA* (no capability of recombination repair, see 2.2.2.4).

*Ps. stutzeri* and *E. coli* were maintained as slope cultures on Nutrient Agar (Oxoid, Basingstoke, U.K.) at 4 °C.

3.1.2: Enumeration of Microorganisms

3.1.2.1: The Miles and Misra Drop Plating Technique

On occasions where single species liquid cultures were counted a version of the Miles and Misra technique was used (Miles and Misra, 1938). The steps involved were as follows:

(1) The sampled liquid was diluted in a series of decimal steps (i.e. $10^{-1}$, $10^{-2}$, $10^{-3}$ etc.). A 0.1 ml aliquot of the culture was added aseptically to 0.9 ml of sterile ¼ strength Ringer solution (Oxoid) in an Eppendorf tube. The tube was closed, and the 1 ml of liquid within was mixed by means of a vortex mixer. Following this, 0.1 ml was withdrawn from the tube, and added aseptically to another Eppendorf tube containing 0.9 ml of sterile Ringer solution. In this manner it was possible to obtain a succession of tenfold dilutions of the original culture.
(2) For each dilution 20 μl drops of Ringer/cell suspension were pipetted aseptically onto dried sterile Tryptone Soya Agar ('TSA'; Oxoid) plates. It was possible to deposit six such drops onto a plate made in a standard (90 mm diameter) petri dish. Each plate was allowed to stand for approximately fifteen minutes to allow the individual drops of liquid to dry onto the agar surface, the plates were then inverted and incubated at 30 °C for 24 hours.

(3) After incubation, the plates were inspected to see at which dilution it was most convenient to count the colonies which had grown on the agar. At low dilutions colony growth was confluent, and it was not possible to obtain counts. Counts were made on plates which had fewer than 40 colonies per drop (10-20 was ideal). When all the plate(s) on which the selected dilution was cultured had been counted, the mean colony count per drop was calculated. This mean was then multiplied by fifty times the dilution factor to give a final count in colony forming units (CFU) per ml.

3.1.2.2: The Spread Plate Technique

In situations where a count of a heterogeneous population of microorganisms in liquid culture was required (e.g. in large-scale cutting oil experiments) a spread plate method was employed.

(1) The liquid sample to be counted was diluted as above, except that 1 ml of sample was added to 9 ml of sterile Ringer solution in a universal bottle.

(2) For each dilution 0.1 ml aliquots of Ringer/cell suspension were pipetted aseptically onto dried sterile TSA plates (one aliquot per plate). A sterile glass cell spreader was used to evenly distribute the liquid over the whole agar plate. The spreader was sterilized before use by immersion in industrial methylated spirits (Fisher Scientific, Loughborough, U.K.) and then passed
quickly through a Bunsen burner flame. The flaming spirit was allowed to burn out before the spreader was cooled. This was achieved by touching the hot spreader twice onto the surface of the agar plate in an area away from that on which the liquid aliquot had been deposited: it was then ready for use. Three aliquots of each dilution were plated. After spreading the plates were inverted and incubated as above.

(3) The mean colony count for the selected dilution was calculated, and the final count (CFU/ml) was determined by multiplying the mean colony count by ten times the dilution factor.

3.1.3: Determination of Regrowth of Metal Working Fluid (MWF) Cultures

The regrowth of the bacterial cultures in the continuously-irradiated MWF experiments (see 5.1.2) was investigated.

After 96 hours of irradiation, a 1 L sample of MWF was removed from the 1000 L batch, and placed in a 2 L vessel. This vessel was transferred to a water bath at 32 °C, and the MWF circulated with a peristaltic pump, in the absence of UV irradiation. The microbial counts (spread plate method) from samples taken over a period of 110 hours were used to construct a growth curve for the culture (see Fig. 5.7).

3.1.4: Identification of Microorganisms Isolated from MWFs

Following enumeration of the heterogeneous microbial population of a commercial oil-water emulsion (Hocut B200, Houghton Vaughan, Birmingham, U.K.) as described above, attempts were made to obtain counts of morphologically distinct colonies. Some of these colonies were isolated for the purpose of identification. The isolates were initially named for the volume
of oil being treated, the time elapsed since treatment began, and a number to
differentiate individual isolates (e.g. 1000/0/001 etc.).

(1) Each selected colony was aseptically transferred onto a sterile Nutrient
Agar slopes in a universal bottle. The slopes were labelled and incubated for
24 hours at 30 °C, after which time the bottle caps were screwed-down tightly
and the slopes stored at 4 °C.

(2) A loopful of each isolate was aseptically transferred into an Erlenmeyer
flask containing 100 ml of sterile Nutrient Broth (Oxoid). The labelled flasks
were incubated in a shaker cabinet for 24 hours at 30 °C, at 150 rpm.

(a) A 0.1 ml aliquot of each resultant culture was aseptically transferred onto a
sterile TSA plate, and spread as in 3.1.2.2. After 24 hours incubation at 30 °C,
the culture grew over the surface of the agar in the form of a 'lawn'.

(b) A drop of each culture was inspected using a light microscope (Model
BHC; Olympus, Tokyo, Japan) with a 100 x oil immersion lens. Note was
taken of the organism type (e.g. bacterium, mould, or yeast), the shape of the
cells, whether or not they occurred in groups or were motile, and of any other
growth features (e.g. pigment production). Visual inspection did not reveal
contaminants in any of the isolates.

(3) The fully grown lawns of isolate cells (2(a) above) were used to provide
material for three further tests:

(a) Gram test (Medical Wire & Equipment, Corsham, U.K.). A paper strip
impregnated with a colourless substrate, L-alanine-4-nitroanilide (LANA),
was wetted with a buffered activating agent. A loopful of fresh culture was
smeared onto the test strip. LANA is hydrolysed by the cell wall peptidases
found in Gram-negative bacteria, producing a bright yellow product,
nitroaniline, within a few minutes. Thus the production of a yellow coloration indicated that a bacterium was Gram-negative, whilst Gram-positive bacteria produced no colour change.

(b) Oxidase test (Pyo-test*, Medical Wire & Equipment). A loopful of culture was smeared onto a test strip impregnated with ascorbic acid and tetramethyl-p-phenylenediamine dihydrochloride (a colourless redox dye). Oxidase positive bacteria will convert the dye to a deep purple product, indophenol blue, within a few minutes. Consequently, oxidase positive bacteria will produce the colour change, whereas oxidase negative bacteria will produce none.

(c) Growth on MacConkey No. 3 Agar (Oxoid). Isolates were streaked-out onto dried plates containing sterile preparations of this agar. The plates were incubated for 24 hours at 37°C. The agar contained lactose and the indicators neutral red and crystal violet. Lactose fermenters, such as the coliform bacilli, will produce intense violet-red colonies when grown on such an agar, whilst non-lactose fermenters are colourless (Collins and Lyne, 1984).

(4) Isolates were classified according to the results of the preceding experiments.

(a) Yeasts and moulds were inoculated onto Sabouraud Dextrose Agar (Oxoid).

(b) Bacteria which were Gram-negative, oxidase positive, motile rods were tested with the API 20NE (bioMérieux UK, Basingstoke, U.K.) identification system for non-enteric bacilli. The resultant numerical profile was used for the identification of the bacterium with the aid of an analytical profile index (Anon., 1988). Any isolates whose profiles could not immediately be matched
were checked on the maker's computer database of profiles. In some cases it was necessary to perform recommended supplementary tests (see (5)).

(c) Bacteria which were Gram-negative and oxidase negative were presumed to be enterobacteria: those which were lactose fermenters were considered to be coliforms.

(5) Tests Supplementary to API 20NE

(a) Lecithinase Production Test. The organism to be investigated was inoculated onto dried egg yolk agar (Harrigan and McCance, 1976) plates, and incubated at 30°C for 3 days. The production of lecithinase is indicated by the presence of an opaque region in the agar adjacent to the region of bacterial growth.

(b) Sorbitol Assimilation Test. The method used was loosely based on that of Stanier et al. (1966).

(i) A base medium was made using phosphate buffered saline ('Dulbecco A'; Oxoid), and 1.0 g/L (NH₄)₂SO₄ (Fisher Scientific).

(ii) Two sets of agar were made: a control batch constituted as described above with the addition of 10 g/L Agar No. 1 (Oxoid), and a test batch, containing in addition 1.0 g/L sorbitol (Sigma Chemicals Ltd., Poole, U.K.).

(iii) Both batches were autoclaved for 10 minutes at 115°C, and cooled to 45°C, before being aseptically poured into sterile petri dishes.

(iv) The agar plates were dried for 45 minutes in a laminar flow cabinet before use.
(v) Stock culture samples of each strain to be tested for sorbitol assimilation were aseptically inoculated onto both the test and control agars. In addition, the same was done with known strains whose abilities to assimilate sorbitol were known.

(vi) The inoculated plates were incubated at 30 °C for 48 hours before being examined for growth, following which they were inoculated for a further 48 hours at the same temperature before growth was again assessed.
3.2: LABORATORY UV INACTIVATION EXPERIMENTS

Bench-top UV inactivation experiments on *Ps. stutzeri* and *E. coli* were carried out in order to obtain dose estimation curves used in bioassay work with liquid bell UV contactors, to investigate the effects of cellular recovery following irradiation, and for the investigation of the effects of hydrogen peroxide/UV treatment.

The methods used for the production of liquid cultures of *Ps. stutzeri* and *E. coli* for experimentation are shown in Fig. 3.1.

**Fig. 3.1: Cell Propagation Procedure**
3.2.1: Apparatus and Methods for Laboratory UV Experiments

3.2.1.1: Pseudomonas stutzeri Bioassay and Repair Work

(1) A 10 ml aliquot of stationary phase culture (see Fig. 3.1) was transferred aseptically into 1 L of sterile Ringer solution (100:1 dilution). The cell counts (of the order of 10^7 CFU/ml) were reduced in this way to levels similar to those found in contaminated cutting oils. The diluted cell suspension can be stored unchanged for several hours in the dark at 4-8 °C.

(2) The 6 W UV lamp (TUV germicidal lamp; Philips, Eindhoven, The Netherlands) specially mounted over a magnetic stirrer unit (see Fig. 3.2) was used. The lamp was switched on and allowed to warm up for 15 minutes. A collimator tube (see Fig. 3.3 (a)) was mounted below the lamp and effectively set the distance between the surface of the sample to be irradiated and the lamp. A shutter, incorporated on the collimator tube, made it possible to expose cell suspensions for precise periods of time. The intensity of the UV light (at wavelength 253.7 nm) at the point corresponding to surface of the cell suspension to be treated was measured using a UVX-25 radiometer (UVP, San Gabriel, CA, U.S.A.).

(3) A 30 ml aliquot of the cell suspension from (1) and a sterilized mini magnetic follower were transferred aseptically to an empty sterile petri dish, and the closed dish was placed on the magnetic stirrer unit beneath the lamp, with the shutter closed. A volume of 30 ml of liquid in a standard size petri dish corresponds to a depth of 5 mm. The magnetic stirrer unit was set so that the follower in the dish was mixing the cell suspension thoroughly whilst avoiding spillage or excessive turbulence.
In order to irradiate the sample, the lid was removed from the petri dish, and the shutter was opened. After the desired irradiation time had elapsed, the shutter was closed and the lid replaced.

The concentrations of viable cells in the irradiated samples were determined using the Miles and Misra drop method (see 3.1.2.1), and compared with the cell concentration in the unirradiated cell suspension so that the survival figures (irradiated cell count + unirradiated cell count) could be calculated.

The light absorbance of the diluted cell suspension at 253.7 nm was measured using a 1 cm path length quartz cuvette (Hellma, New York, NY, U.S.A.) in a Shimadzu (Kyoto, Japan) UV-1201 spectrophotometer. If the depth of suspension, the incident UV intensity, and the absorbance of the suspension are known the average UV intensity in the irradiated sample can be calculated (Morowitz, 1950). Dose was calculated by multiplying the exposure time by the average intensity.

3.2.1.2: *Escherichia coli* 11190 Bioassay Work

A strain of *E. coli* deficient in repair mechanisms (*uvrA*, *recA*) was used in cases where anticipated exposure to UV was such as to produce low doses.

The procedure was the same as in 3.2.1.1, except that:

1. A 10 ml aliquot of stationary phase culture (see Fig. 3.1) was aseptically added to 500 ml of sterile Ringer solution (50:1 dilution) to produce the cell suspension for treatment.

2. A frame holding a PTFE UV intensity-reducing filter (see Fig 3.3 (b)) was attached to the irradiation apparatus, the lamp and filter being lowered to a
position only a few centimetres above the surface of the cell suspension. The entire irradiation process was carried out in the dark.

(3) The UV intensity was measured by potassium ferrioxalate actinometry (see 3.6.1). A 60 ml volume of actinometry fluid was placed in a petri dish and irradiated for a set time period.
Fig. 3.2: Bench Top Irradiation Apparatus

Retort stand

6 W low pressure UV lamp in housing with moveable shutter

(Lamp height: variable)

Petri dish with cell suspension and magnetic follower

Magnetic stirrer unit

UV driver

To power source

Fig. 3.3: Detail of Collimator (a) and of UV Intensity-Reducing Filter (b)

(a) Collimator tube

Rotating shutter

(b) PTFE sheet filter

Filter holder

Rotating shutter
3.3: CELLULAR REPAIR EXPERIMENTS

In order to investigate the effects of repair *Ps. stutzeri* suspensions were first prepared and irradiated with far UV light (253.7 nm) as shown in Fig. 3.2. Further steps in these experiments were as follows:

(1) A sample was taken for enumeration immediately after far UV irradiation, in order to determine the number of survivors.

(2) Aliquots of 2 ml volume from the far UV-treated sample were aseptically transferred into sterile, thin-walled glass sample bottles. The sealed sample bottles were then placed in the photoreactivation apparatus (see Fig. 3.4). This apparatus consists essentially of a glass-bottomed water bath which was irradiated from below by an 18W blue-black near UV light source (Model ‘TL’ D 18V/08; Philips) with peak output between 360 and 370 nm. The base of the reactivation unit comprised two 5 mm thick glass sheets, separated by a space 10 mm wide. This space formed a cell which was filled with 1M CuSO₄ (Fisher Scientific), to filter out light of wavelengths below 313 nm emitted by the source (Calvert and Pitts, 1966). These have been shown to induce additional damage to cells (Harm, 1980). The whole unit was placed in a chilled cabinet so that the water bath temperature could be maintained between 4 and 6 °C. Holding under these conditions would have essentially arrested growth, but would allow repair to proceed. In order to determine the extent of dark repair processes (rather than photoreactivation), control sample tubes which had been made opaque by covering with aluminium foil were also used. Samples were held as described for one hour, as preliminary investigations had shown that maximum cell reactivation occurred within this period. The serial dilution, plating and counting of the bacteria was as described in 3.1.2.1.
Fig. 3.4: Photoreactivation Apparatus

3.4: EXPERIMENTS USING BELL CONTACTORS

Fig. 3.5 is a cutaway view of the type of nozzle used to produce liquid bells during the course of the work. The liquid enters through a side arm and travels downward in the annular compartment created by the space between the outer and middle tubes. At the bottom of the nozzle the annulus of liquid exits via the nozzle aperture, impacting on the striking plate as it does so. A thin bell-shaped liquid sheet is formed as the liquid travels outward under its momentum, falling at the same time. The middle tube houses a component to which the striking plate is attached. This assembly is attached to the inner tube, and is capable of vertical movement inside the middle tube. This arrangement allows the nozzle aperture to be altered. At the top end of the middle tube a circular plug is fitted, through which the inner tube passes. The inner tube (and thus the striking plate) is fastened in position by means of a grub screw which passes through the plug. The inner tube also provides a route via which air may be pumped into the bell.

Fig. 3.5: The Bell Nozzle
UV disinfection experiments were carried out using three pieces of apparatus, one of which was partly constructed as a prototype before the project began.

3.4.1: The Prototype Apparatus

Figure 3.6 shows the general layout of the prototype apparatus. The UV source was a single, centrally-mounted, 6-8 W low pressure mercury arc tube (Starna Lamps, Chadwell Heath, U.K.). A stainless steel cone was mounted at the base of the lamp, onto which the bell impinged during operation. In contrast to later equipment, there was no constant head tank, the liquid being pumped directly to the nozzle.

The liquid flow rate was maintained between 8 and 9 L/min, whilst the flow rate of compressed air to the centre of the bell was 6.75 L/min. The aperture between the end of the bell nozzle and the striking plate was 1.7 mm. The volume of liquid in the whole system was 10 litres. The temperature was
maintained in the range 20-25°C by recirculation of the liquid through the cooling tank.

Some initial work was done in this small prototype apparatus on the disinfection of cutting oil emulsions. Aerated cultures of *Ps. stutzeri* were cultured at 25°C in 3% v/v DCM 24-2 (Houghton Vaughan) water-soluble metalworking fluid, 5 litres of culture being held in a 10 litre container. When a culture was to be irradiated it was placed in the tank, and diluted with a further 5 litres of fresh 3% DCM 24-2 oil. Oil samples for bacterial counts were taken before irradiation, and at set periods during any one experiment. Where it was desired to observe the effect of sequential irradiations on regrowth, 5 litres of oil from the contactor tank were taken for cultivation at the end of the experiment.

3.4.2: The Laboratory-Scale Apparatus

Fig. 3.7 is a schematic of the layout of the laboratory-scale apparatus. The source was a centrally mounted 6-8 W low pressure mercury arc (experimental prototype; Starna Lamps). The lamp was positioned in a closed reactor chamber consisting of a section of borosilicate glass piping (L = 50 cm, D = 30 cm; QVF, Corning, Stone, U.K.) with two circular PVC end plates. The whole apparatus was designed to provide an environment relatively free of outside contamination for experimentation on homogeneous suspensions of *Ps. stutzeri* cells. The pump used was a peristaltic model (AB1 SA; Rossi Motoriduttori, Modena, Italy), and the tank was a 45 L narrow-necked plastic carboy.
The UV dose in the laboratory-scale apparatus was estimated by bioassay with *Ps. stutzeri* and *E. coli* suspensions and chemically with the potassium ferrioxalate liquid actinometer (see 3.6.1). In both cases liquid was pumped past the light (after it had been lit for 15 minutes) in the form of a bell, and samples were collected after the liquid had passed the lamp once only (i.e. single pass). The dose estimation with potassium ferrioxalate had to be carried out in the absence of all other light.

Experiments were also conducted using this contactor on *Ps. stutzeri* suspensions treated by recycle. These were UV disinfection experiments with differing volumes of suspension in order to investigate volume effects.

All cell suspensions were prepared by 100:1 dilution of stationary phase liquid culture with 0.7% w/v sterile saline.
3.4.3: The Pilot-Scale Apparatus

A much larger contactor was also constructed for the purpose of investigating the effects of continuous UV treatment on relatively large volumes of contaminated cutting oil emulsion (up to 1 m³). Fig. 3.8 shows the arrangement of the apparatus for these experiments. As the volumes of oil treated were relatively large, the collecting vessel beneath the UV lamps was lower than the liquid level in the main tank. It was necessary, therefore, to fit a smaller receiving tank from which treated oil was pumped back into the main tank for recycle. In addition to a centrally-mounted UV lamp, sixteen lamps were evenly located around the outside of the bell. These radially-mounted lamps were connected electrically as four banks of four lights each. The central lamp, as well as each separate bank of radial lamps, was capable of being controlled independently. The lamps used were 42 W low pressure mercury arcs, Type TSL/PH (Voltarc, Fairfield, CT, U.S.A.).

It was found that, for the relatively high flow rates employed in work with this apparatus, the liquid bell was not quite as stable as in the laboratory-scale apparatus, and further that the introduction of air into the centre of the bell had no discernible effect. Accordingly, the practice was abandoned.
The radial lamps were held in a removable carousel (see Fig. 3.9). This comprised two circular PVC rings. These rings were joined to each other to form an open cylinder by six PVC supporting struts. A sheet of highly polished anodised aluminium was 'wrapped' on the inside of the struts to form an enclosed cylinder. Such sheeting reflects about 85% of incident short wave UV light (Phillips, 1983). There were sixteen holes in the top plate, through which the lamps and their quartz thimbles were located. The lamps were kept in position by individual cups attached to the top side of the bottom plate, into which the rounded ends of the thimbles fitted. The whole carousel assembly was attached to the collecting vessel by means of a flange.
The type of oil used in the experiments was Hocut B200 (Houghton Vaughan). The concentrate was diluted to 4% w/v with ‘synthetic’ hard water (see 3.6.3.1). Volume losses caused by evaporation during the experiments were made up by the addition of distilled water. Different volumes of cutting oil were continuously irradiated by recycle past all seventeen lamps.

Oil temperature and pH were recorded during the course of the experiments, and microbial counts were made at regular intervals. At the beginning of each run the fresh oil was inoculated with bacterial strains isolated from spoilt cutting oil samples provided by P. Carpenter (Houghton Vaughan). As each run progressed, bacteria and fungi were isolated for later identification (see 3.1.4). In order to estimate the reduction of UV intensity caused by fouling of the quartz thimbles, the intensity of the central lamp was measured before and after each run with a UVX-25 radiometer.

The UV dose generated by the apparatus was estimated by single pass bioassays with *Ps. stutzeri*, and by potassium ferrioxalate actinometry.
3.4.4: Hydraulic Salt Tracer Tests

Salt tracer studies were performed in the laboratory-scale apparatus in order to investigate the hydraulic characteristics of this type of contactor.

**Fig. 3.10: Configuration for Salt Tracer Tests**


Fig. 3.10 shows the arrangement of the laboratory-scale apparatus for salt tracer studies. The contactor was operated in continuous flow mode with the water from the bell passing to drain via a conductivity cell.

The steps in the process were as follows:

(1) The bell nozzle aperture and the control valve were set to produce a stable bell at a known flow rate. Next 5 ml of 200 g/L NaCl (Fisher Scientific) were rapidly injected via a syringe into the water to produce a 'spike' of salt solution in the flow. The salt solution was injected perpendicular to the axis of flow upstream of the inlet to the bell nozzle (see Fig. 3.10).
(2) The salt solution in the effluent from the contactor passed out of the bell reactor chamber via a conductivity cell (Model 2041/400; Kent Industrial Measurements Ltd., Chertsey, U.K.), and thence to drain. The cell was connected to a chart recorder (Model PM 8251; Philips) via a conductivity meter (Model PTI-20; WPA Scientific Instruments, Cambridge, U.K.). The time taken from injection of the tracer solution to the appearance of the resultant peak was also recorded.

(3) The process was repeated three times for each different bell flow rate. In every case the chart recorder paper speed was 300 mm/min.

3.5: THE POWER OUTPUT OF UV LAMPS AT 253.7 nm

Where it was necessary to determine directly the output of a particular model of UV lamp the following procedure was followed:

(1) Lamp length (excluding the end caps of the lamp) was measured.

(2) The lamp was set up in a darkened room, switched on, and left for 15 minutes in order to achieve constant emission.

(3) The intensity of UV irradiation at 253.7 nm was determined at a variety of points relative to the lamp. In some cases this was done by using a UVX-25 radiometer, in others by use of a timed exposure of potassium ferrioxalate actinometer fluid (see 3.6.1). The intensity of the lamp was measured perpendicular to its long axis.

(4) Measurements were taken as is shown in Fig. 3.11.
Fig. 3.11: Measurements Taken to Calculate Lamp Power Output

L = length of radiating portion of lamp.
R = radial distance of point of intensity measurement from long axis of lamp.
Z = vertical distance of point of intensity measurement from baseline.
X = vertical distance of beginning of radiating portion of lamp from baseline.
P = point of intensity measurement.

(5) By substituting into the expression (Esplugas et al., 1983):

\[ I(R, Z) = \frac{S_l}{4\pi R} \left\{ \arctan \left( \frac{Z - X}{R} \right) - \arctan \left( \frac{Z - X - L}{R} \right) \right\} \] (3-1)

where \( I = \) intensity, \( W/m^2 \), it was possible to calculate \( S_l \), the energy emission per unit time and unit length in units \( W/m \). By multiplying \( S_l \) by the lamp length (\( L \), in metres) a figure for the lamp power output (\( W \)) at 253.7 nm was obtained.

3.6: ANALYTICAL METHODS

3.6.1: Potassium Ferrioxalate Actinometry

Since potassium ferrioxalate is a sensitive actinometer for a wide range of light wavelengths, all preparation for, and use of, the chemical in UV-measuring experiments has to be done in darkness or under far-red safelights. The experimental procedures involved were as follows:
(1) Bulk quantities of 0.006 M (2.947 g/L) potassium ferrioxalate, K$_3$Fe(C$_2$O$_4$)$_3$, (Pfaltz & Bauer Inc., Waterbury, CT, U.S.A.), 0.05 M in H$_2$SO$_4$ (Fisher Scientific), were made-up in a large tanks near to the respective pieces of apparatus. The system was adjusted so that liquid flowing through the bell nozzle went straight to drain instead of being recycled. The actinometer solution was poured manually into the header tank, and, as soon as a liquid bell had been formed, the UV lamps were lit. Samples of actinometer solution which had thus been exposed to a single pass through the contactor (for different irradiation regimes in the laboratory and pilot-scale equipment) were collected. Also taken were samples from the stock tank, both before and after the experiments had taken place.

(2) From each sample a 10 ml aliquot was pipetted into a 25 ml volumetric flask. To this was added 2 ml of 0.1% w/v aqueous 1,10 phenanthroline hydrate, C$_{12}$H$_8$N$_2$H$_2$O (Fisher Scientific), and 5 ml of buffer. The flask was made-up to 25 ml with distilled water, mixed thoroughly, and left for 30 minutes to develop. The buffer was made by dissolving 600 ml M sodium acetate, CH$_3$COONa (Fisher Scientific), in distilled water in a 1 L volumetric flask, and adding 360 ml 0.5 M H$_2$SO$_4$, before making up to one litre with distilled water.

(3) The absorbance of the resultant mixture at the wavelength 510 nm was measured in a 1 cm cuvette, using a spectrophotometer (Model SPG-250; Pye Unicam, Cambridge, U.K.). The contents of a flask made-up as in (2), but in which 10 ml of unirradiated actinometer solution was substituted for the 10 ml sample aliquot, were used for the absorbance blank.

(4) The measurable photoproduct formed when potassium ferrioxalate solutions were exposed to light is the ferrous (Fe$^{2+}$) ion. Ferrous ions complex with phenanthroline, producing an orange-coloured solution with a maximum absorbance wavelength of 510 nm. Thus the concentration of
ferrous ions in an irradiated potassium ferrioxalate sample can be calculated from its absorbance at 510 nm as long as the molar absorption coefficient of the complexed solution (with respect to ferrous ion concentration) is known. The molar absorption coefficient, $\varepsilon$, for the spectrophotometer was determined by developing solutions of known ferrous ion concentration with phenanthroline, and plotting absorbance against concentration. It is known from Beer's Law that absorbance equals $\varepsilon$cl (where $c$ = the concentration of the absorbing solution in moles/L, and $l$ = cuvette path length in cm). As the path length of the cuvette used in this case was 1 cm, the slope of such a plot will equal $\varepsilon$. The value of $\varepsilon$ was $1.083 \times 10^4$ L/mole.cm. It follows that the concentration of ferrous ions can be calculated by dividing the absorbance of the developed sample solution by this figure. Since all the ferrous ions present in the 25 ml of developed solution come from the 10 ml portion of potassium ferrioxalate test solution which was added to it, the molar concentration of ferrous ions given for the 25 ml of developed solution must be multiplied by 2.5 to give the correct figure.

(5) The UV dose can be calculated from figures for the concentration of ferrous ions produced by using the following relation:

$$
\text{Dose (in mW-s/cm}^3\text{)} = \frac{[\text{Fe}^{2+}]_u - [\text{Fe}^{2+}]_b}{\Phi} \times (4.719 \times 10^8) \times \frac{1}{1000} 
$$

(3-2)

where $[\text{Fe}^{2+}]_u =$ concentration of $\text{Fe}^{2+}$ ions in exposed solution in moles/L, $[\text{Fe}^{2+}]_b =$ concentration of $\text{Fe}^{2+}$ ions in unexposed solution (in this case, the average of the concentrations of the pre- and post-irradiation stock tank solution figures was used), $\Phi = 1.26$ (the quantum yield, moles $\text{Fe}^{2+}$/Einstein; Lee and Seliger, 1964), $4.719 \times 10^8 =$ the energy of one mole of photons of UV light of wavelength 253.7 nm (in mW-s/Einstein), dividing by 1000 merely changes the dosage units from mW-s/L to mW-s/cm$^3$. 
3.6.2: Corrosion Tests

Corrosion tests were performed on diluted Hocut B200 (4% v/v in water) both before and during treatment with UV light. The method is based on the International Petroleum Standard Method IP 287/83 (Institute of Petroleum, 1987).

3.6.2.1: Preparation of Cutting Oil Dilutions

The metalworking concentrate to be examined (Hocut B200) was diluted to 4% with synthetic hard water of 200 ppm as calcium carbonate hardness. This was prepared by dissolving 0.3449 g/L of calcium sulphate dihydrate, CaSO₄·2H₂O, (Fisher Scientific) in distilled water.

3.6.2.2: Experimental Procedure

(1) Cast iron chips (P.E.R.A., Melton Mowbray, U.K.) were washed in acetone (Fisher Scientific), and dried for 5-10 minutes in an oven at 105°C. They were used immediately.

(2) The chips were then sieved in a 850 μm mesh sieve, and the fines and any chips which showed signs of rust were rejected.

(3) A centrally positioned 35 mm square was drawn in pencil on a piece of filter paper (Whatman No. 3; Whatman, Maidstone, U.K.). The paper was then placed in a 90 mm petri dish, and a circular former (a plastic sheet with a 35 mm square cut out of its centre) placed over it.

(4) With the aid of the former and a plastic spatula, 1 g of the dried chips were distributed evenly in a single layer over the square test area on the filter paper.
(5) A 2 ml aliquot of the metalworking fluid to be examined was pipetted onto the chips, care being taken to distribute the liquid evenly over the chips on the plate.

(6) After 2 hours the chips were removed from the filter paper, which was washed with distilled water and then dried for later examination.

(7) The test area of the filter paper was scanned to a * . TIF graphics file using a Hewlett Packard ScanJet 3c scanner (Boise, ID, U.S.A.) attached to an IBM-compatible computer equipped with Hewlett Packard DeskScan II software (version 2.2). The image of each test region thus generated was examined by means of the Visilog (version 4.1.3; Noesis, Vélizy, France) image analysis programme, which allowed the total test area and the area discoloured by corrosion to be measured in arbitrary units so that the percentage of the test region marked by corrosion could be easily calculated.

3.6.3: Measurements of pH, Density and Surface Tension

(1) Measurements of pH were all made using a PH-20 electrode (Ashby Technical Products Ltd., Ashby-de-la-Zouch, U.K.) calibrated with pH 4 and pH 7 standard buffer solutions (Fisher Scientific).

(2) Measurements of density were made using a standard glass density bottle, with distilled water as the reference.

(3) Measurements of surface tension were made using an automatic DuNouy-type ring detachment meter (Model DB 2kS; White Electrical Instrument Co. Ltd., Malvern, U.K.).
CHAPTER 4: BIOASSAY AND ACTINOMETRY UV DOSE ESTIMATION

4.1: INTRODUCTION

A knowledge of the dose applied to a liquid undergoing UV irradiation is important in optimising the design of contactors and ultimately, in assessing the economic viability of any UV treatment process. Two different techniques, bioassay and actinometry, were employed here to determine the UV dose delivered to liquids being irradiated in the form of liquid bells. The principles behind both of these methods were fully described in 3.2.1 and 3.6.1, but briefly, bioassay techniques are based on determining the survival of a suitable test organism following irradiation and then relating this to dose. Initially, the bacterium Ps. stutzeri was used but a repair deficient E. coli strain, 11190, was employed in later studies.

Actinometry, on the other hand, relies on the detection of chemical changes brought about by light and the conversion of these to doses from a knowledge of the quantum yield of the light-catalysed reaction. The potassium ferrioxalate actinometer was selected for use here because it absorbs a high percentage of incident light at the wavelength of interest (253.7 nm) for small liquid path lengths (Calvert and Pitts, 1966). This was of particular importance as the liquid sheets constituting the liquid bells were typically less than 0.5 mm thick and were irradiated by UV for periods less than one second (Shama et al., 1996). Therefore it was essential that detectable changes were induced in the actinometer by such short exposure times.

Dose measurements were conducted using both the laboratory scale contactor, which was fitted with one central UV lamp, and the pilot-scale apparatus which was provided with 17 lamps. This latter arrangement permitted UV dose measurements to be carried out with different radiation
regimes. Each apparatus was operated at more than one flow rate for at least one of the dose estimation methods used. In addition, a UV radiometer was used to measure UV intensities directly and attempts were made to estimate UV doses from estimates of liquid residence times in the vicinity of the UV lamps.

4.2: RESULTS

4.2.1: Pseudomonas stutzeri Bioassay

The *Ps. stutzeri* dose-response curve (Fig. 4.1) was created by fitting a polynomial regression curve to the experimental data obtained by exposing suspensions of the organism to a UV lamp of known intensity for fixed periods of time as described in 3.2.1.1. The results are combined from two separate groups of irradiation experiment, both of which used different stock bacterial suspensions. The mirror image of the dose-response curve (Fig. 4.2) was fitted by the inverse function of that for survival in terms of dose. This expression is given below:

\[
y = -0.072x^3 - 0.599x^2 - 3.071x + 0.867
\]

(4-1)

where \(x = \log_{10} \) fractional survival, and \(y = \) UV dose in mW-s/cm\(^2\).
Fig. 4.1: The *Ps. stutzeri* Dose-Response Curve

![Graph showing the Ps. stutzeri Dose-Response Curve with experimental groups 1 & 2 indicated.](image)

Fig. 4.2: Reflection of *Ps. stutzeri* Dose-Response Curve in x=y

![Graph showing the reflection of the Ps. stutzeri Dose-Response Curve in the x=y plane.](image)
The experimental procedure for dose estimation involved altering the configuration of the contactor so that the cell suspension once irradiated was not returned to the feed tank (see 3.7). Table 4.1 shows the data obtained by the exposure of a suspension of *Ps. stutzeri* cells to the single UV lamp in the laboratory-scale apparatus. The apparatus was operated at two flow rates, 650 and 860 litres per hour. Once fractional survival values had been ascertained, the survival-dose expression (4-1) was applied to obtain the dose estimate.

Table 4.1: Single Pass Dose Estimates For The Laboratory-Scale Apparatus Using *Ps. stutzeri* Bioassay

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>N/N₀</th>
<th>Dose (mW-s/cm²)</th>
<th>Flow rate (L/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>0.56</td>
<td>1.61</td>
<td>860</td>
</tr>
<tr>
<td>1B</td>
<td>0.60</td>
<td>1.52</td>
<td>860</td>
</tr>
<tr>
<td>2A</td>
<td>0.88</td>
<td>1.04</td>
<td>860</td>
</tr>
<tr>
<td>2B</td>
<td>0.63</td>
<td>1.46</td>
<td>860</td>
</tr>
<tr>
<td>3A</td>
<td>0.51</td>
<td>1.72</td>
<td>650</td>
</tr>
<tr>
<td>3B</td>
<td>0.46</td>
<td>1.84</td>
<td>650</td>
</tr>
<tr>
<td>4A</td>
<td>0.16</td>
<td>2.97</td>
<td>650</td>
</tr>
<tr>
<td>4B</td>
<td>0.18</td>
<td>2.85</td>
<td>650</td>
</tr>
<tr>
<td>5A</td>
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<td>2.75</td>
<td>650</td>
</tr>
<tr>
<td>5B</td>
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<td>650</td>
</tr>
<tr>
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<td>0.50</td>
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<td>650</td>
</tr>
<tr>
<td>6B</td>
<td>0.50</td>
<td>1.74</td>
<td>650</td>
</tr>
<tr>
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<td>0.32</td>
<td>2.25</td>
<td>650</td>
</tr>
<tr>
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<td>2.28</td>
<td>650</td>
</tr>
<tr>
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<td>2.70</td>
<td>650</td>
</tr>
</tbody>
</table>

(* The numbers refer to separately prepared cell cultures. The letters afterward to replicate measurements made using the same batch of cells.)

Table 4.2 shows data for the exposure of *Ps. stutzeri* suspension to single passes through the pilot-scale apparatus. A feature of this apparatus was that it was equipped with multiple UV sources and it was possible to irradiate the liquid bell produced by different numbers and configurations of UV sources. Experiments were conducted with all 17 lamps on, with the 16 lamps external to the bell on, or with just the single central lamp on. The apparatus was
operated at flow rates of both 990 and 1080 litres per hour. The survival-dose relationship used was the same as applied in the previous section.

Table 4.2: Single Pass Dose Estimates For Pilot-Scale Apparatus Using *Ps. stutzeri* Bioassay

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>N/N₀</th>
<th>Dose (mW-s/cm²)</th>
<th>Flow rate (L/hr.)</th>
<th>Lamps Lit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>0.42</td>
<td>1.94</td>
<td>990</td>
<td>ALL</td>
</tr>
<tr>
<td>1B</td>
<td>0.35</td>
<td>2.15</td>
<td>990</td>
<td>ALL</td>
</tr>
<tr>
<td>2A</td>
<td>6.25E-3</td>
<td>5.49</td>
<td>990</td>
<td>ALL</td>
</tr>
<tr>
<td>2B</td>
<td>6.90E-2</td>
<td>3.74</td>
<td>990</td>
<td>ALL</td>
</tr>
<tr>
<td>3A</td>
<td>3.2E-2</td>
<td>4.36</td>
<td>1080</td>
<td>ALL</td>
</tr>
<tr>
<td>3B</td>
<td>3.7E-2</td>
<td>4.25</td>
<td>1080</td>
<td>ALL</td>
</tr>
<tr>
<td>3C</td>
<td>2.91E-3</td>
<td>5.97</td>
<td>1080</td>
<td>OUTSIDE ONLY</td>
</tr>
<tr>
<td>3D</td>
<td>2.65E-3</td>
<td>6.03</td>
<td>1080</td>
<td>OUTSIDE ONLY</td>
</tr>
<tr>
<td>3E</td>
<td>0.23</td>
<td>2.60</td>
<td>1080</td>
<td>INSIDE ONLY</td>
</tr>
<tr>
<td>3F</td>
<td>0.21</td>
<td>2.70</td>
<td>1080</td>
<td>INSIDE ONLY</td>
</tr>
<tr>
<td>4A</td>
<td>6.79E-4</td>
<td>6.87</td>
<td>1080</td>
<td>ALL</td>
</tr>
<tr>
<td>4B</td>
<td>1.50E-3</td>
<td>6.38</td>
<td>1080</td>
<td>ALL</td>
</tr>
<tr>
<td>4C</td>
<td>7.50E-5</td>
<td>8.38</td>
<td>1080</td>
<td>OUTSIDE ONLY</td>
</tr>
<tr>
<td>4D</td>
<td>8.57E-5</td>
<td>8.28</td>
<td>1080</td>
<td>OUTSIDE ONLY</td>
</tr>
<tr>
<td>4E</td>
<td>0.24</td>
<td>2.56</td>
<td>1080</td>
<td>INSIDE ONLY</td>
</tr>
<tr>
<td>4F</td>
<td>0.20</td>
<td>2.75</td>
<td>1080</td>
<td>INSIDE ONLY</td>
</tr>
</tbody>
</table>

(*The numbers refer to separately prepared cell cultures. The letters afterward to replicate measurements made using the same batch of cells. ALL = all 17 lamps lit, OUTSIDE ONLY = the 16 circumferentially-arranged lamps lit, INSIDE ONLY = the single centrally-mounted lamp only lit.*)
4.2.2: *Escherichia coli* 11190 Bioassay

Fig. 4.3 shows the dose-response curve for *E. coli* 11190, whilst Fig. 4.4 shows the inverse of the dose-response curve. The results are combined from three separate groups of irradiation experiments, each of which used different stock bacterial suspensions. The polynomial expression for dose in terms of survival was obtained by the same method as was that for *Ps. stutzeri* and is given below:

\[
y = 4.174x^2 - 17.441x + 0.577 \quad (4-2)
\]

where \( x = \log_{10} \) fractional survival, and \( y \) = UV dose in \( \mu W\text{-s/cm}^2 \).
Fig. 4.3: *E. coli* 11190 Dose-Response Curve

![Graph showing the dose-response curve for E. coli 11190.](image)

- ■, ○, △ = Experimental groups 1–3

Fig. 4.4: Reflection of *E. coli* 11190 Dose-Response Curve in x-y

![Graph showing the reflection of the dose-response curve in x-y coordinates.](image)
It is immediately apparent that the dose-response curve of *E. coli* does not display the shoulder which was a feature of the *Ps. stutzeri* data. Moreover, *E. coli* 11190 is clearly more sensitive to UV than is *Ps. stutzeri*; an applied dose of 90.5 μW-s/cm² resulted in a four log reduction in viable cell counts compared with the 6.6 mW-s/cm² (more than 72 times greater) required to produce the same result for *Ps. stutzeri*.

Table 4.3 shows the dose estimates obtained using the *E. coli* 11190 bioassay for a series of experiments conducted using the laboratory scale apparatus at a fixed flowrate of 650 litres/hr.

**Table 4.3: Single Pass Dose Estimates for Laboratory-Scale Apparatus Using E. coli 11190 Bioassay**

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>N/N₀</th>
<th>Dose (μW-s/cm²)</th>
<th>Dose (mW-s/cm²)</th>
<th>Flow rate (L/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>2.50E-4</td>
<td>117.55</td>
<td>0.118</td>
<td>650</td>
</tr>
<tr>
<td>1B</td>
<td>2.16E-4</td>
<td>120.59</td>
<td>0.121</td>
<td>650</td>
</tr>
<tr>
<td>2A</td>
<td>2.46E-5</td>
<td>169.63</td>
<td>0.170</td>
<td>650</td>
</tr>
<tr>
<td>2B</td>
<td>1.86E-5</td>
<td>176.48</td>
<td>0.176</td>
<td>650</td>
</tr>
<tr>
<td>3A</td>
<td>5.25E-3</td>
<td>62.03</td>
<td>0.062</td>
<td>650</td>
</tr>
<tr>
<td>3B</td>
<td>5.45E-3</td>
<td>61.44</td>
<td>0.061</td>
<td>650</td>
</tr>
</tbody>
</table>

(* The numbers refer to separately prepared cell cultures. The letters afterward to replicate measurements made using the same batch of cells.)

**4.2.3: Potassium Ferrioxalate Actinometry**

Table 4.4 shows the data obtained after the exposure of batches of an acidified 0.006M potassium ferrioxalate solution to single passes through the laboratory-scale apparatus at a flowrate of 650 litres/min. The experimental procedures and calculations involved were described in detail in 3.6.1. Calculations produced dose values in a volumetric form (e.g. mW-s/cm³), and in order to enable comparisons to be made with the bioassay-derived doses, it was necessary to convert the former into the more commonly used unit area form (e.g. mW-s/cm²). In order to achieve this, it was necessary to use the concept of mean irradiated depth (Harris *et al.*, 1987a). Mean irradiated depth
can simply be defined as the total volume of liquid in a contacting apparatus divided by the liquid surface area exposed to irradiation. The values of bell (inside) surface area, volume, Mean Irradiated Depth, nozzle gap and residence time for each flow rate used in both pieces of apparatus are given in Appendix C.2. Conversion was achieved by multiplying the volumetric dose by the mean irradiated depth. Table 4.4 shows the actinometric doses for the laboratory scale contactor at a flowrate of 650 litres/min, whilst Table 4.5 displays the data obtained using the pilot-scale apparatus at flowrates of 990 and 1080 litres/min.

Table 4.4: Single Pass Dose Estimates For The Laboratory-Scale Apparatus Based On Actinometric Measurements

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>Lamp lit?</th>
<th>Absorbance (510 nm)</th>
<th>Volumetric Dose (mW-s/cm²)</th>
<th>Area Dose (mW-s/cm²)</th>
<th>Flow Rate (L/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>NO</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>650</td>
</tr>
<tr>
<td>1B</td>
<td>NO</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>650</td>
</tr>
<tr>
<td>1C</td>
<td>YES</td>
<td>0.05</td>
<td>4.32</td>
<td>0.11</td>
<td>650</td>
</tr>
<tr>
<td>1D</td>
<td>YES</td>
<td>0.15</td>
<td>13.07</td>
<td>0.34</td>
<td>650</td>
</tr>
<tr>
<td>1E</td>
<td>YES</td>
<td>0.08</td>
<td>6.92</td>
<td>0.18</td>
<td>650</td>
</tr>
<tr>
<td>2A</td>
<td>NO</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>650</td>
</tr>
<tr>
<td>2B</td>
<td>YES</td>
<td>0.14</td>
<td>11.24</td>
<td>0.29</td>
<td>650</td>
</tr>
<tr>
<td>2C</td>
<td>YES</td>
<td>0.16</td>
<td>12.97</td>
<td>0.33</td>
<td>650</td>
</tr>
<tr>
<td>2D</td>
<td>YES</td>
<td>0.15</td>
<td>12.10</td>
<td>0.31</td>
<td>650</td>
</tr>
<tr>
<td>3A</td>
<td>NO</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>650</td>
</tr>
<tr>
<td>3B</td>
<td>YES</td>
<td>0.15</td>
<td>11.24</td>
<td>0.29</td>
<td>650</td>
</tr>
<tr>
<td>3C</td>
<td>YES</td>
<td>0.17</td>
<td>12.97</td>
<td>0.33</td>
<td>650</td>
</tr>
<tr>
<td>3D</td>
<td>YES</td>
<td>0.15</td>
<td>11.24</td>
<td>0.29</td>
<td>650</td>
</tr>
</tbody>
</table>

(* The numbers refer to separately prepared cell cultures. The letters afterward to replicate measurements made using the same batch of cells.)
Table 4.5: Single Pass Dose Estimates for the Pilot-Scale Apparatus Based On Actinometric Measurements

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>Lamps lit</th>
<th>Absorbance (510 nm)</th>
<th>Volumetric Dose (mW-s/cm²)</th>
<th>Area Dose (mW-s/cm²)</th>
<th>Flow Rate (L/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>NONE</td>
<td>0.29</td>
<td>0</td>
<td>0</td>
<td>990</td>
</tr>
<tr>
<td>1B</td>
<td>NONE</td>
<td>0.17</td>
<td>0</td>
<td>0</td>
<td>990</td>
</tr>
<tr>
<td>1C</td>
<td>ALL</td>
<td>1.04</td>
<td>70.03</td>
<td>1.36</td>
<td>990</td>
</tr>
<tr>
<td>1D</td>
<td>INSIDE ONLY</td>
<td>0.73</td>
<td>43.23</td>
<td>0.84</td>
<td>990</td>
</tr>
<tr>
<td>1E</td>
<td>INSIDE ONLY</td>
<td>1.04</td>
<td>70.03</td>
<td>1.36</td>
<td>990</td>
</tr>
<tr>
<td>2A</td>
<td>NONE</td>
<td>0.47</td>
<td>0</td>
<td>0</td>
<td>1080</td>
</tr>
<tr>
<td>2B</td>
<td>NONE</td>
<td>0.47</td>
<td>0</td>
<td>0</td>
<td>1080</td>
</tr>
<tr>
<td>2C</td>
<td>ALL</td>
<td>1.16</td>
<td>59.65</td>
<td>3.49</td>
<td>1080</td>
</tr>
<tr>
<td>2D</td>
<td>ALL</td>
<td>0.99</td>
<td>44.95</td>
<td>2.63</td>
<td>1080</td>
</tr>
<tr>
<td>2E</td>
<td>OUTSIDE ONLY</td>
<td>1.41</td>
<td>81.27</td>
<td>4.75</td>
<td>1080</td>
</tr>
<tr>
<td>2F</td>
<td>OUTSIDE ONLY</td>
<td>1.25</td>
<td>67.43</td>
<td>3.94</td>
<td>1080</td>
</tr>
<tr>
<td>2G</td>
<td>INSIDE ONLY</td>
<td>1.23</td>
<td>65.70</td>
<td>3.84</td>
<td>1080</td>
</tr>
<tr>
<td>2H</td>
<td>INSIDE ONLY</td>
<td>1.20</td>
<td>63.11</td>
<td>3.69</td>
<td>1080</td>
</tr>
</tbody>
</table>

(* The numbers refer to separately prepared cell cultures. The letters afterward to replicate measurements made using the same batch of cells. ALL = all 17 lamps lit, OUTSIDE ONLY = the 16 circumferentially-arranged lamps lit, INSIDE ONLY = the single centrally-mounted lamp only lit.)

4.3: DISCUSSION

The dose response curve displayed by *Ps. stutzeri* (Fig. 4.1) is of the ‘classic’ shouldered type characteristic of many organisms (Harm, 1980). Different interpretations have been put forward as to the true significance of the shouldered portion of the curve.

‘Multitarget’ or ‘multihit’ models explain shouldered curves in terms of classical target theory. Multitarget curves may be expected where suspended cells are normally found in clumps (and therefore where all individuals must be inactivated to achieve the loss of the ability to form a colony), or where cells contain multiple sets of their genetic material (this condition, known as polyploidy, is encountered in some protozoans, but is not important when the inactivation of bacteria, viruses, or fungi is considered). It has been noted that closely related *E. coli* mutants, which would not be expected to have markedly differing numbers or types of targets, have significantly different survival
characteristics following UV exposure (Haynes, 1964). Some mutants produce shouldered curves, whereas others show more or less simple exponential inactivation.

The shoulders on the survival curves of the more resistant strains have also been interpreted as demonstrating the ability these cells have to repair radiation damage. The shoulder of the curve thus represents the dose range within which the cells can repair the damage incurred. This type of curve becomes steep and linear at higher dosage levels, at this point the repair mechanisms may themselves have been inactivated, or the total number of lesions in the microbial DNA may have overrun the ability of the repair systems to cope with the damage produced. Since cellular repair mechanisms for both UV-induced and general DNA damage are now well-known, it seems that the repair mechanism explanation of shouldered survival curves is more credible than considering the effect to be due to the presence of more than one UV susceptible area. This view is reinforced by the fact that bacterial mutants which are known to be repair deficient (e.g. E. coli 11190) exhibit survival curves that lack an initial shoulder.

The slope of the dose response curve of Ps. stutzeri (Fig. 4.1) decreased at relatively high values of applied dose. This ‘tailing’ of the curve has been attributed either to shielding of cells by suspended particles or other cells, or to the survival of individuals within cell clumps (Jagger, 1967; Harm, 1980). However, microscopic investigation of the test suspensions used here revealed neither cell clumping nor suspended solids (see Chapter 5), and the absorption coefficient of the cell suspension employed was not very great, so that it was unlikely that cells could have shielded each other from incident UV.

Other workers have used the dose-survival curves of various organisms for bioassay work. Braunstein et al. (1996) used the MS2 coliphage, which has a
linear dose-response relationship (i.e. there is no shoulder or tailing). *Bacillus subtilis* (ATCC 6633) spores have been employed in bioassay tests by at least two groups (Qualls and Johnson, 1983; Harris *et al*., 1987a); both found that the dose-response curve of the spores was of sigmoidal form (as was that of *Ps. stutzeri*). However, both groups ignored the shoulder and tailing portions of the *B. subtilis* curve, electing to fit a linear regression relation to the exponential region. In both cases it was fortunate that the bioassay results generated corresponded to the exponential portion of the organism’s dose-survival curve.

The presence in the dose-response curve of plateau regions, such as shoulders and tails, can have a pronounced effect on dose determination, as small variations in the measurement of surviving fraction will yield large variations in equivalent doses. This effect is compounded when surviving fraction is plotted on a logarithmic scale. For *Ps. stutzeri* these plateau regions correspond to doses less than 3.0 and greater than 12 mW-s/cm². Table 4.2 shows that all of the dose estimates made using the laboratory scale apparatus, and many from the pilot scale apparatus fall within the region where estimation becomes unreliable. This realisation led to the search for an organism which would display a more linear dose response curve. *E. coli* 11190 was selected because it is both dark and recombination repair deficient (*uvrA*, *recA*). Although a polynomial expression was fitted to the dose response data (Fig. 4.4) to enable dose estimates to be made with the greatest possible precision, the data is seen to be well characterised by a straight line when plotted in semi-log form ('exponential inactivation', as shown in Fig. 4.3). The mechanistic interpretation of this behaviour is that inactivation of these cells follows first order kinetics:

\[
\ln \frac{N}{N_0} = -kD \tag{2-2}
\]
In other words, the rate of decrease of active absorbing organisms with respect to dose is dependent only on the total number of active particles at any time. This implies that the total number of photons previously absorbed is unimportant, and that any one photon can cause inactivation. It can be concluded, therefore, that, when a straight line dose-survival curve is obtained on a semilog plot, inactivation is a 'single-hit single-target' process (Harm, 1980) This means that one photon only is required to hit (that is, be absorbed by and inactivate) the target site within the absorbing cell.

Crucially, linear dose-response behaviour enabled dose estimates to be made with greater precision. The values obtained using the *E. coli* bioassay are significantly, some 95% lower, than those obtained using the *Ps. stutzeri* for the laboratory-scale contactor.

Point readings of intensity were made using a radiometer with the probe located approximately in the position of the liquid film constituting the bell in the laboratory scale contactor. Although the readings obtained were approximate, they can be used to provide a test of consistency with the data obtained using actinometry and bioassays if the dose estimates produced by the latter (see table 4.6) were to be converted to intensity estimates. This was done by applying the simple relationship:

\[
\text{Intensity} = \frac{\text{Dose}}{\text{Residence time}}.
\]

Residence times were obtained as explained in 6.4.1.4. The results are presented in Table 4.7. The actinometric and the *E. coli* bioassay dose estimates (1.27 and 0.55 mW/cm² respectively) agree broadly with the point radiometer measurement (2.00 mW/cm²). The mean estimate from the *Ps. stutzeri* bioassay (10.84 mW/cm²) seems rather high, since it would be expected that the average intensity in an absorbing system to be somewhat
less than that registered when the UV output travels only through air (i.e. to the radiometer).

Table 4.6: Summary of Dose Estimates (D, mW·s/cm²) for Laboratory-Scale Apparatus

<table>
<thead>
<tr>
<th></th>
<th>650 L/hr</th>
<th></th>
<th>860 L/hr</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>D</td>
<td>σₐ₁</td>
<td>n</td>
</tr>
<tr>
<td><em>Ps. stutzeri</em> bioassay</td>
<td>11</td>
<td>2.34</td>
<td>±0.51</td>
<td>4</td>
</tr>
<tr>
<td><em>E. coli</em> 11190 bioassay</td>
<td>6</td>
<td>0.12</td>
<td>±0.05</td>
<td>-</td>
</tr>
<tr>
<td>Potassium ferrioxalate</td>
<td>9</td>
<td>0.27</td>
<td>±0.08</td>
<td>-</td>
</tr>
<tr>
<td>Pot. ferriox. (corrected)†</td>
<td>-</td>
<td>0.26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Radiometer*</td>
<td>1</td>
<td>0.43</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

(* point measurement at the approximate bell radius from the central lamp, using a UVX-25 radiometer to measure intensity. This could be used to obtain an approximate estimate of dose using the relationship: dose = intensity × residence time. † Potassium ferrioxalate data corrected for the 5% of light output which is visible and non-germicidal (Phillips, 1983). n = number of replicates, σₐ₁ = small population standard of deviation.)

Table 4.7: Summary of Intensity Estimates (I, mW/cm²) for Laboratory-Scale Apparatus

<table>
<thead>
<tr>
<th></th>
<th>650 L/hr</th>
<th></th>
<th>860 L/hr</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>I</td>
<td>σₐ₁</td>
<td>n</td>
</tr>
<tr>
<td><em>Ps. stutzeri</em> bioassay</td>
<td>11</td>
<td>10.84</td>
<td>±2.38</td>
<td>4</td>
</tr>
<tr>
<td><em>E. coli</em> 11190 bioassay</td>
<td>6</td>
<td>0.55</td>
<td>±0.24</td>
<td>-</td>
</tr>
<tr>
<td>Potassium ferrioxalate</td>
<td>9</td>
<td>1.27</td>
<td>±0.36</td>
<td>-</td>
</tr>
<tr>
<td>Pot. ferriox. (corrected)†</td>
<td>-</td>
<td>1.21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Radiometer*</td>
<td>1</td>
<td>2.00</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

A summary of dose estimates for the pilot scale contactor, operated under various irradiation arrangements, using actinometry and the *Ps. stutzeri* bioassay is given in Table 4.8. In all cases, the doses obtained using the bioassay are higher than those generated by actinometric measurements. A partial explanation for this is that low pressure mercury arc lamps ('germicidal lamps'), although considered to be virtually monochromatic sources, do not emit solely at 253.7 nm. This wavelength is very close to the main biologically destructive wavelength, which is organism dependent, but lies in the region of 250 to 266 nm (see 2.1). Even wavelengths emitted by the UV lamps which do not exert a lethal effect on micro-organisms will induce changes in the ferrioxalate actinometer. It would be possible to obtain an
estimate of the extent to which the non-germicidal wavelengths influence actinometric measurements by further shrouding the UV sources with tubing made from borosilicate glass, which would absorb UV light whilst allowing the longer wavelengths to induce changes to the actinometer. The doses obtained with and without the borosilicate shrouds could then be subtracted from one another to enable more realistic comparisons to be made with bioassay dose estimates. In the absence of experimental data of this nature, recourse can be made to published estimates of the percentage of the light output of a low-pressure mercury arc lamp which is visible and therefore non-germicidal. Phillips (1983) gives a value for this type of light of approximately 5% of total light output. Using these estimates, the actinometric measurements were corrected (by multiplication by 0.95) and are shown for completeness in Tables 4.6-4.9. It should be remembered, however, that the calculations required by the actinometric method use a quantum value of 1.26 for all incident light (assumed to be of wavelength 253.7 nm); accurate measurements of the quantum yield of potassium ferrioxalate for the full spectrum of light emitted from low pressure mercury arc lamps would be useful when using the method for UV dose estimation.

Harris and co-workers (1987a) also found that their potassium ferrioxalate dose estimates were consistently lower than those obtained by means of a (B. subtilis) bioassay when account was taken of reflectance, but were much greater (2-5 times) when reflectance was not taken into account. In the aforementioned work, the irradiated surface area of the treated liquid was calculated as the total surface area of the conduits of the reactors where 100% reflectance was assumed. Where no reflectance was assumed, the irradiated surface area was calculated as the surface area of liquid facing the lamps. This type of treatment is acceptable for the annular tube-type reactors used in that work, but is not relevant in the case of a water bell irradiated from both sides.
Table 4.8: Summary of Dose Estimates (D) for the Pilot-Scale Apparatus

<table>
<thead>
<tr>
<th>Lamps Lit</th>
<th>990 L/hr</th>
<th>1080 L/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>D</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>3.33 ±1.65</td>
</tr>
<tr>
<td>OUTSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>INSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

Ps. stutzeri bioassay

<table>
<thead>
<tr>
<th>Potassium ferrioxalate</th>
<th>990 L/hr</th>
<th>1080 L/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>I</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>12.71 ±6.29</td>
</tr>
<tr>
<td>OUTSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>INSIDE ONLY</td>
<td></td>
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</tr>
</tbody>
</table>

Potassium ferrioxalate

<table>
<thead>
<tr>
<th>Pot. ferriox. (corrected)†</th>
<th>990 L/hr</th>
<th>1080 L/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>I</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>4.93</td>
</tr>
<tr>
<td>OUTSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>INSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

(ALL = all 17 lamps lit, OUTSIDE ONLY = the 16 circumferentially-arranged lamps lit, INSIDE ONLY = the single centrally-mounted lamp only lit. †, D, n and σ_m as Fig. 4.6, p. 84.)

Table 4.9: Summary of Intensity Estimates (I) for the Pilot-Scale Apparatus

<table>
<thead>
<tr>
<th>Lamps Lit</th>
<th>990 L/hr</th>
<th>1080 L/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>I</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>12.71 ±6.29</td>
</tr>
<tr>
<td>OUTSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>INSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

Ps. stutzeri bioassay

<table>
<thead>
<tr>
<th>Potassium ferrioxalate</th>
<th>990 L/hr</th>
<th>1080 L/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>I</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>5.19</td>
</tr>
<tr>
<td>OUTSIDE ONLY</td>
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<td>2</td>
</tr>
<tr>
<td>INSIDE ONLY</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Pot. ferriox. (corrected)†

<table>
<thead>
<tr>
<th>Pot. ferriox. (corrected)†</th>
<th>990 L/hr</th>
<th>1080 L/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>I</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>4.93</td>
</tr>
<tr>
<td>OUTSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>INSIDE ONLY</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

(ALL = all 17 lamps lit, OUTSIDE ONLY = the 16 circumferentially-arranged lamps lit, INSIDE ONLY = the single centrally-mounted lamp only lit. I = mW/cm².)

Using the data obtained at a flowrate of 1080 L/hr (for which most data are available) it is possible to rank the doses delivered as follows:-

All lamps < Inside lamp only < Outside lamps only (actinometry)

Inside lamp only < All lamps < Outside lamps only (Ps. stutzeri bioassay).

Although the sample sizes were small, the data were analysed statistically using the t-Test method for the comparison of the means of small samples.
(Bailey, 1981). It was assumed that the unknown variances were not equal. There were only two potassium ferrioxalate measurements for each case, and it was not possible to show any significant difference between any of the lighting regimes. For the bioassay measurements, however, the results for the case where the inside lamp only was lit differ from the outside lamps only and all lamps configurations at the 5% significance level. This was not the case when outside lamps only and all lamps were compared.

The dose estimation results for the pilot-scale apparatus appear somewhat anomalous as the measured dose does not increase with the number of lamps being used. However, both dose estimation methods reveal an apparent consistency in that the dose was highest where the bell was irradiated by the lamps arranged round the circumference of the bell only. Further, the doses delivered when the bell was irradiated by the single axially-positioned lamp and by all of the lamps simultaneously were very similar. Differences in the dose estimates obtained for the three lighting regimes are not statistically significantly; further comparisons are unlikely to prove useful at this stage, therefore.

Dose estimates derived from the actinometric procedures employed here (see Tables 4.6 and 4.7) were more consistent for each sample group than those obtained by either of the two bioassays. Although the accuracy of the actinometric method is well documented (Calvert and Pitts, 1966; Harris et al., 1987a), there are some inherent difficulties which must be addressed.

Doses are obtained in volumetric form (i.e. mW-s/cm$^3$). In order to convert to the more commonly used unit area form (i.e. mW-s/cm$^2$) it is necessary to use the concept of Mean Irradiated Depth (e.g. volumetric dose $\times$ mean irradiated depth = area dose). This is a simple matter for symmetrical reactors (e.g. annular types), where the surface area of the contactor and thickness of liquid passing the UV source (and thus the effective volume of the contactor) are
constant. Determining the Mean Irradiated Depth for a bell contactor is not as straightforward because the thickness of the liquid film comprising the bell varies with vertical distance from the nozzle (Shama et al., 1996). However, in order to calculate the Mean Irradiated Depth, it was necessary to ascertain the radius of the bell at regular vertical distances from the nozzle. Initially this was achieved by photographic measurement, but all bell radius data presented in this work were obtained by means of a model for the surface of the bell (see Chapter 6). The corresponding liquid sheet thickness values (h) were also calculated by the model, thus making it possible to plot both the inside and outside surfaces of the bell graphically. Polynomial regression curves fitted to these plots made it possible to obtain functions which described these surfaces. The volume of revolution of each function was obtained by means of the standard formula:

\[ \text{Volume} = \int \pi f(x)^2 \, dx \quad (4-3) \]

The volume of the bell was calculated by subtracting the volume of revolution of the inside surface of the bell from that of the outside surface. The area of a surface of revolution was calculated as follows:

\[ \text{Area} = \int 2\pi f(x) \sqrt{1 + (f'(x))^2} \, dx \quad (4-4) \]

For irradiation by a single axially located lamp it was a relatively simple task to calculate the Mean Irradiated Depth. The molar extinction coefficient of 0.006M potassium ferrioxalate is 5.11x10³ L.mol⁻¹.cm⁻¹ (own measurement and calculation). This implies that a 0.5 mm thick liquid film will absorb over 97% of incident light at 253.7 nm. Therefore, it is easier to assume that the effect of the irradiation of the bell from both sides is the same as that of the resultant liquid sheet being irradiated from one side only by the same number of lights. The mean irradiated depth for multilamp operation in the pilot-scale apparatus is therefore calculated by dividing the bell volume by the area of
one of the surfaces only. It should be noted that the changes in bell thickness and the aforementioned uncertainties related to surface area render Mean Irradiated Depth a less useful quantity then when it is used in relation to conventional symmetrical solid-wall contactors.

Furthermore, dose estimates obtained using ferroxalate actinometers are susceptible to the presence of extraneous light and also to the presence of ferrous ions in the apparatus. The ferroxalate actinometer displays sensitivity to a wide range of light wavelengths and it was necessary to carry out all the required manipulations under darkened conditions, with far red photographic safelights being the only acceptable sources of light under which work can be conducted. The use of experimental 'blanks' (samples of actinometer which had been circulated around the apparatus before the sources were lit) avoided the possibility of false dose estimates due to the presence of either of these two factors. Such procedures were relatively simple to perform using the laboratory scale contactor but presented considerable practical difficulties when applied to the pilot scale unit.

By comparing the fractional survival figures for both bioassays (Tables 4.2, 4.3 and 4.5) and the absorption figures for the potassium ferroxalate actinometry (Tables 4.6 and 4.7), it can be seen that results were more consistent within one experiment than they were across all experiments for the same supposed conditions. This suggests that the actual dose may have varied from experiment to experiment. Actinometric dose determinations were carried out with a single batch of potassium ferroxalate and every precaution was taken to ensure that the potassium ferroxalate solutions were made up in an identical manner. The possibility remains that the photo-induced conversion of ferric to ferrous ions was adversely affected by some inhibitory substance(s) present in the contactors, although no specific interfering compounds have previously been reported. All of the lamps were conditioned before use by being left alight continuously for 72 hours in
accordance with the manufacturers instructions. Conditioning in this way is claimed by the manufacturers to result in constancy of emission. As a further precaution, the lamps were allowed to warm-up for the same period of time before each experiment in order to ensure steady state emission. Moreover, the quartz tubes shrouding the lamps was cleaned before each experiment so that variations in lamp output could not be attributed to dirt or dried films on the shrouds. The spectral output of low pressure mercury vapour lamps is a function of temperature and the efficacy of commercial lamps has been shown to be affected by ambient temperature (Phillips, 1983). A likely cause of the observed variability in dose estimates could be attributed to ambient temperature effects.

An alternative method of dose estimation, based on a mathematical model of liquid bells coupled with a UV emission model, is described in Chapter 6.

4.4: CONCLUSIONS

UV dose estimates were obtained for pilot and laboratory scales of UV contactor using potassium ferrioxalate actinometry and bioassays based on Ps. stutzeri and a repair-deficient strain of E. coli.

There was good agreement between the estimates produced using the E. coli 11190 bioassay and the potassium ferrioxalate actinometer. This was attributed to the fact that E. coli displayed linear dose-response characteristics. Further evidence for the validity of dose estimates based on these two methods was provided by calculating doses using intensity estimates obtained with a UV radiometer. The estimates from the Ps. stutzeri bioassay were much higher than those produced by all the other methods evaluated. This was because the doses measured did not coincide with the linear portion of the organisms’ dose-response curve.
Estimates for the dose applied by the pilot-scale apparatus did not rise linearly when the number of UV sources used was increased. Comparison of dose estimates for different irradiation regimes showed that did not differ to a statistically significant degree, and it is apparent that further experimental studies are required to verify the results presented here.

Whilst good agreement was obtained with identical batches of potassium ferrioxalate solutions, variations occurred between batches. It was suggested that this might have been caused either by the presence of chemical species affecting the conversion of ferric ions or alternatively, that the observed variations were due to the effects of ambient temperature on the spectral emission of the UV lamps.

Despite the measures which were taken to ensure that test suspensions of *Ps. stutzeri* and *E. coli* were generated and handled with consistency, variations between batches of cells were nevertheless apparent. These difficulties could possibly be overcome by using spores which might be expected to display less variability than vegetative cells, but in order to enable dose estimates to be made with precision, an absolute requirement would be that the spores display well characterised dose response characteristics.
CHAPTER 5: IRRADIATION OF METAL WORKING FLUIDS

5.1: INTRODUCTION

The thin film contactor had been identified earlier as offering particular advantages for the UV treatment of liquids which are strongly UV-absorbing. Metal working fluids (MWFs) fall into this category. MWFs are used industrially as workpiece cooling and lubrication fluids (e.g. on lathes). Most MWFs are essentially oil-water emulsions, although they may contain many additional components. These include anti-corrosion agents, emulsifiers and biocides. The latter are added in order to control the growth of microorganisms which commonly contaminate MWFs and by so doing, impair the performance of the emulsion. As explained in 2.5.3.1, there is considerable interest in industry in developing alternative, physical methods of contamination control. MWFs present a considerable challenge to UV-based disinfection processes as oil-water emulsions not only absorb, but also scatter, UV light very strongly. Another feature of MWF use in industry is the wide range of system volumes which exist. These can vary from scores of litres to tens of thousands of litres. This is significant, because UV disinfection technology might ultimately prove to be economically viable over a restricted range of volumes yet the scale of demand for the technology could still prove to be appreciable.

In this chapter experiments are described in which MWFs (formulated without biocides) were artificially contaminated with both pure and mixed cultures of bacteria and then irradiated with UV using thin film contactors. In the initial experiments a small prototype contactor equipped with a single UV lamp was used but in later work a larger multi-lamp unit was employed to treat volumes of MWF ranging from 200 to 1000 L over periods of several days. In addition to monitoring heterotrophic plate counts during irradiation, attempts were made to determine the fate of individual contaminating species
of micro-organisms. Also investigated was population re-growth and the ability of one species, *Ps. stutzeri*, to repair UV-induced damage.

### 5.2: RESULTS

#### 5.2.1: Periodic Irradiation of Infected MWF

The MWF used, DCM 24-2 (Houghton-Vaughan), was of the type normally employed for general cutting applications in metalwork. This type of MWF comprises essentially a mineral oil and fatty acid base, with lesser amounts of other components (see 2.5.1). The DCM 24-2 formulation did not include a biocide. The manufacturers recommended dilution with water to a concentration in the range of 5-10% by volume before use; in this case a dilution of 4% was used. The oil emulsion was deliberately infected by inoculation with *Pseudomonas stutzeri*.

Fig. 5.1 shows the change in numbers of the microbial population in the infected MWF with time. The emulsion was exposed to periodic UV irradiation in a prototype contactor (like the laboratory-scale apparatus, this had only one axially-mounted low pressure UV source). In each case the period of irradiation (started at points A-D respectively) was of two hours duration.
Fig 5.1: Periodic UV Irradiation of Metal Working Fluid (DCM 24-2) Infected with *Pseudomonas stutzeri*
5.2.2: Continuous Treatment of Bulk Volumes of MWF

The continuous irradiation experiments were carried out using three volumes of the test liquid: 200, 400 and 1000 L. Originally, 200 L of 4% Hocut B200 were prepared, and inoculated with strains of *Ps. fluorescens* and *Shewanella putrefaciens* which had previously been isolated from spoilt cutting oils. For the remaining two experiments the volume was made up to new level required (400 and 1000 L respectively), and microbial levels allowed to build-up from the small number of survivors from the previous disinfection experiment. Prior to irradiation, the contents of the tank were circulated for 2 hours in every 24 to prevent the onset of anaerobic conditions.

In order to assess the feasibility of the industrial application of the bell contactor to the treatment of MWFs, a series of long-running experiments were conducted involving the treatment of relatively large volumes of microbially-infected emulsion. The data presented in this section detail the effects of the irradiation on the total microbial population and pH of the MWF. The fluid used was Hocut B200, diluted to 4% with artificially-prepared water of known hardness (in order to eliminate any of the variation that can be caused by this, see 2.5.2).

Inspection of the graphs for the continuous irradiation of the metalworking fluid (Figs. 5.2-5.4) shows a relatively rapid initial decline in microbial population (during the first 6-8 hours for the 200 and 400 L experiments, Figs. 5.2 and 5.3; and during the first 24 hours for the 1000 L experiment, Fig. 5.4) to a steady low level of infection, in one case even to an apparent state of sterility. The first experiment (on a 200 L volume of emulsion) had to discontinued due to accidental leakage, but in the second and third experiments the total cell count was reduced to a consistently very low level (0-1000 CFU/ml). Measurements of pH during the course of the experiments
showed an almost linear reduction, from a starting point close to 7.0, at a rate approximately equal to one complete unit in every 100 hours.

In all experiments (Figs. 5.3 and 5.4) the temperature of the emulsion rose above ambient temperature into the low-mid thirties Celsius. The temperature increase was due to thermal input by the centrifugal pumps used to circulate the liquid and also to the UV sources.

In addition to recording the effects of UV irradiation on the number of living cells in the contaminated oil samples, counts were also recorded for morphologically distinct types of microbial colony. The individual isolate counts were obtained by counting the number of colonies which were visually similar to the isolated colony. This technique was not without error, but was of use as an approximate guide to gross changes in the composition of the microbial population. Representative colonies were isolated so that they could be characterised with a battery of microbiological tests. The tests carried out on individual microbial isolates have been detailed earlier (see 3.1.4). Any organism which was found to be a Gram negative, oxidase positive motile rod was suitable for testing with the API20NE test system. Where large numbers of colonies were isolated and extensive testing using the API20NE system became impractical, Gram negative, oxidase positive, non-lactose fermenting motile rods were recorded as Pseudomonas spp. Additionally, it was possible to identify Ps. aeruginosa by sight after isolation, because of the production of the distinctive chloroform-soluble blue pigment pyocyanin. The results of the microbiological tests carried out on the organisms isolated from the test liquid are shown in Tables 5.1-5.3, and reveal that the majority of the isolates belonged to the genus Pseudomonas. Samples of infected Hocut B200 were also inspected by means of a light microscope in order to determine if any cell clumping was occurring.
Samples of MWF taken during all three large scale irradiation experiments were placed in the reactivation apparatus described in 3.3 and maintained either under near UV illumination or in the dark for periods of up to 4 hours. Aliquots were plated out every 2 hours to determine whether repair of UV-mediated cell damage was occurring. With the exception of the results for the 400 L experiment after 4 hours in the dark, bacterial numbers decreased with time. The data from these experiments, as well as the fractional survival figures for the MWF after a single pass through the contactor, are shown in Table 5.4.

At 96 hours following the commencement of irradiation of the 1000 L experiment, a 1 L sample of MWF was removed and placed in a 2 L vessel. This vessel was rapidly transferred to a water bath at 32 °C and the MWF circulated with a peristaltic pump, in the absence of UV irradiation. Samples were taken over a period of 110 hours and plated out in order to monitor the regrowth of the microbial population (see Fig. 5.7). The figure shows that the population reached a steady concentration approximately 80 hours after transfer.
Fig. 5.2: Continuous Treatment of 200L Hocut B200

$N_0 = 1.0 \times 10^7$ CFU/ml, ■ = Fractional Survival, ● = pH.
Fig. 5.3: Continuous Treatment of 400L Hocut B200

$N_0 = 1.0 \times 10^7$ CFU/ml, ■ = Fractional Survival, ● = pH, ◦ = Temp.
Fig. 5.4: Continuous Treatment of 1000L Hocut B200

$N_0 = 4.3 \times 10^6$ CFU/ml, ■ = Fractional Survival, ● = pH, ◆ = Temp.
### Table 5.1: Microbiological Tests on Isolates from 200L Experiment

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Isolate</th>
<th>Cell Type</th>
<th>Gram</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Lactose Ferm.</th>
<th>Pyocyanin Prod.</th>
<th>API20NE</th>
<th>CFU/ml</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200/001</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>6.0E6</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>0</td>
<td>200/002</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>4.0E6</td>
<td>UNID Gram +ve</td>
</tr>
<tr>
<td>0</td>
<td>200/003</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>5.0E6</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>0</td>
<td>200/004</td>
<td>yeast</td>
<td>NA</td>
<td>NT</td>
<td>+/-</td>
<td>-</td>
<td>NT</td>
<td>3.0E5</td>
<td>UNID yeast</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>200/005</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.0E3</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>25</td>
<td>200/006</td>
<td>tetrad</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>NT</td>
<td>8.0E3</td>
<td>UNID Gram +ve</td>
</tr>
<tr>
<td>51</td>
<td>200/007</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.3E5</td>
<td>UNID Gram +ve</td>
</tr>
<tr>
<td>51</td>
<td>200/008</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>3.2E5</td>
<td>Ps. aeruginosa</td>
</tr>
</tbody>
</table>

NA = not applicable, NT = not tested.

### Table 5.2: Microbiological Tests on Isolates from 400L Experiment

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Isolate</th>
<th>Cell Type</th>
<th>Gram</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Lactose Ferm.</th>
<th>Pyocyanin Prod.</th>
<th>API20NE</th>
<th>CFU/ml</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>400/001</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>Ps. aeruginosa</td>
</tr>
<tr>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.0E6</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>6</td>
<td>400/003</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.1E5</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
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<td>400/004</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>2.0E4</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
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<td>400/005</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.8E4</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>30</td>
<td>400/006</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>3.7E4</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>54</td>
<td>400/007</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>5.0E4</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>54</td>
<td>400/008</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>4.0E4</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>72</td>
<td>400/009</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.0E3</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>96</td>
<td>400/010</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.0E3</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>96</td>
<td>400/011</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>4.0E3</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>150</td>
<td>400/012</td>
<td>coccoid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>50</td>
<td>UNID Gram +ve</td>
</tr>
<tr>
<td>150</td>
<td>400/013</td>
<td>tetrad</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>30</td>
<td>UNID Gram +ve</td>
</tr>
<tr>
<td>174</td>
<td>400/014</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>9.0E2</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>222</td>
<td>400/015</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>1.4E2</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>222</td>
<td>400/016</td>
<td>coccoid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>10</td>
<td>UNID Gram +ve</td>
</tr>
<tr>
<td>222</td>
<td>400/017</td>
<td>dicoccoid</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>1.0E2</td>
<td>UNID Gram +ve</td>
</tr>
</tbody>
</table>

NT = not tested.
Table 5.3: Microbiological Tests on Isolates from 1000L Experiment

<table>
<thead>
<tr>
<th>Time (hr.)</th>
<th>Isolate</th>
<th>Cell Type</th>
<th>Gram</th>
<th>Motility</th>
<th>Oxidase</th>
<th>Lactose Ferm.</th>
<th>Pyocyanin Prod.</th>
<th>API20NE</th>
<th>Lecithinase</th>
<th>Sorbitol Ass.</th>
<th>CFU/ml</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000/001</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>2.0E6</td>
<td>Ps. putida</td>
</tr>
<tr>
<td>0</td>
<td>1000/002</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>5.0E6</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>6</td>
<td>1000/003</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>1.3E6</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>6</td>
<td>1000/004</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>8.0E5</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>31</td>
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<td>filamentous fungus</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>1.0E4</td>
<td>UNID mould</td>
</tr>
<tr>
<td>31</td>
<td>1000/006</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>3.0E4</td>
<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
<td>48</td>
<td>1000/007</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>2.0E3</td>
<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
<td>52</td>
<td>1000/008</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>5.0E3</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>52</td>
<td>1000/009</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
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<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
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<td>1000/010</td>
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<td>-</td>
<td>NT</td>
<td>NT</td>
<td>4.0E3</td>
<td>Ps. putida</td>
</tr>
<tr>
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<td>1000/011</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>2.0E3</td>
<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
<td>76</td>
<td>1000/012</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>1.0E3</td>
<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
<td>76</td>
<td>1000/013</td>
<td>rod</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>6.0E3</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>76</td>
<td>1000/014</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>3.0E3</td>
<td>Pseudomonas paucimobilis</td>
</tr>
<tr>
<td>76</td>
<td>1000/015</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>3.0E4</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>100</td>
<td>1000/016</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>1.15E3</td>
<td>Ps. putida</td>
</tr>
<tr>
<td>145</td>
<td>1000/017</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>9.0E2</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>145</td>
<td>1000/018</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>4.0E7</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>151</td>
<td>1000/019</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>NT</td>
<td>1.8E3</td>
<td>Ps. aeruginosa</td>
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<tr>
<td>169</td>
<td>1000/020</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>90</td>
<td>UNID Gram -ve</td>
</tr>
<tr>
<td>169</td>
<td>1000/021</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>20</td>
<td>Ps. fluorescens</td>
</tr>
<tr>
<td>169</td>
<td>1000/022</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>2.4E3</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>192</td>
<td>1000/023</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>3.0E3</td>
<td>Ps. putida</td>
</tr>
<tr>
<td>192</td>
<td>1000/024</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>2.8E3</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>215</td>
<td>1000/025</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>10</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>215</td>
<td>1000/026</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>2.3E2</td>
<td>Ps. aeruginosa</td>
</tr>
<tr>
<td>219</td>
<td>1000/027</td>
<td>rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>20</td>
<td>UNID mould</td>
</tr>
<tr>
<td>247</td>
<td>1000/028</td>
<td>filamentous fungus</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>1.0E4</td>
<td>UNID mould</td>
</tr>
</tbody>
</table>

NA = not applicable, NT = not tested.
Figures 5.5 and 5.6 show the numbers of those bacterial species which were identified after isolation. Data from the 200 L experiment are not displayed graphically because of its premature ending. The total microbial count data in Figures 5.5 and 5.6 agree generally with those used to calculate the fractional survival data plotted in Figs. 5.2-5.4, but differ in the range +69 to -18 %. This is partly because the former was estimated from counts on a single agar plate for any particular time in any of the experiments (the number of each colony type being also noted), whereas the fractional survival data are based on counts from three plates at each data point.

In a situation where it is impractical to isolate every colony from every agar plate some anomalies will inevitably occur. For instance, it is unlikely that *Ps. putida* comprised the whole microbial population in the 1000 L experiment at 100 hours given the presence of both *Ps. aeruginosa* and *Ps. fluorescens* before and after this time (see Fig. 5.6). However, it is apparent that *Ps. aeruginosa* and *Ps. fluorescens* were the two commonest organisms encountered in the Hocut B200 system. *Shewanella putrefaciens*, which with *Ps. fluorescens*, was used inoculate the initial 200 L of emulsion, was never subsequently encountered. This is probably because the strain used could not survive in Hocut B200, or could not compete with other microorganisms which established themselves. *Ps. fluorescens* was present during all three experiments (see Table 5.1, Figs. 5.5 and 5.6), being especially important in the early stages. In the 200 L experiment (see Table 5.1) *Ps. fluorescens* appeared to be eliminated rapidly from the MWF. In the 400 L experiment (see Fig. 5.5) it apparently disappeared suddenly after 30 hours, and in the 1000 L experiment (see Fig. 5.6) its numbers declined rapidly after 50 hours, and it was present at 169 hours in very low numbers. *Sphingomonas paucimobilis* (a bacterium closely allied to, and once classified as a part of, the genus *Pseudomonas*) was isolated only between the 35th and 100th hours of the 1000 L experiment (Fig. 5.6), but nevertheless in relatively high numbers. *Ps. aeruginosa* was the organism isolated most frequently in the continuous
irradiation experiments (accounting for 17 of the 53 isolates made), it was a major contributor to microfloral numbers in all cases.
Fig. 5.5: Population Sizes for Isolates from 400L Experiment

![Graph showing microbial count over time for isolates from 400L experiment. The graph displays the microbial count (CFU/ml) on a logarithmic scale against time (hours) from 0 to 150. The graph includes symbols for total count, Ps. aeruginosa, and Ps. fluorescens.]
Fig. 5.6: Population Sizes for Isolates from 1000L Experiment

- Total count
- *Ps. aeruginosa*
- *Ps. fluorescens*
- + *Ps. putida*
- Sphingomonas paucimobilis
Table 5.4: Fractional Survival Data for Single Pass and Reactivation Experiments

<table>
<thead>
<tr>
<th>Fractional Survival (N/N₀)</th>
<th>200 L</th>
<th>400 L</th>
<th>1000 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Pass</td>
<td>0.80</td>
<td>0.77</td>
<td>0.92</td>
</tr>
<tr>
<td>Dark holding 2 hours</td>
<td>0.68</td>
<td>0.58</td>
<td>0.75</td>
</tr>
<tr>
<td>Light holding 4 hours</td>
<td>0.51</td>
<td>0.90</td>
<td>-</td>
</tr>
<tr>
<td>Light holding 2 hours</td>
<td>0.80</td>
<td>0.53</td>
<td>0.64</td>
</tr>
<tr>
<td>Light holding 4 hours</td>
<td>0.57</td>
<td>0.52</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 5.7 shows the regrowth of the microbial population of a sample of the MWF from the 1000 L experiment following UV irradiation. The curve fitted to the data points is of the classic population growth-type. The straight line has been fitted to the linear portion of the curve to enable the specific growth rate (μ, the slope of this line) to be calculated for later use in inactivation modelling (see Chapter 7).

Fig. 5.7: Regrowth of a Mixed Cell Culture in Hocut B200 Oil Emulsion Following UV Irradiation

![Graph showing regrowth of a mixed cell culture](image)

Fig. 5.8 (a) and (b) reveals the extent of fouling by MWF of the quartz thimbles shrouding the UV lamps following 247 hours of irradiation. The
figures show that the extent of fouling was not uniform and also that fouling was more pronounced on the lower part of the lamps. On termination of the irradiation experiments, close examination of the lamps revealed that a dark brown film had been deposited on the lower part of the lamps. Radiometer measurements were used to estimate the approximate reduction in UV intensity caused by the film (see Table 5.5). An average estimate was recorded of approximately 65% intensity reduction.

Fig. 5.8: Fouling on External UV Sources After 247 Hours Irradiation
Table 5.5: Estimates of UV Intensity Reduction Due to Fouling

<table>
<thead>
<tr>
<th>Intensity (mW/cm²)</th>
<th>Expt.</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400 L</td>
<td>7.8</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>1000 L</td>
<td>7.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Readings with UVX-25 radiometer, 15 cm from lit central source, after 222 and 247 hours continuous irradiation respectively.

Corrosion tests were conducted on samples from each of the three pilot-scale experiments. The cast iron chip method (see 3.6.2) was used. Briefly, this method involved contacting cast iron chips, which were evenly distributed on a filter paper, with the MWF sample. After 2 hours, the chips were removed from the filter paper, which was then washed with distilled water and dried. Any corrosion of the chips occurring within the test period was apparent as rust stains on the filter paper. The area of the test square covered by rust staining was determined and expressed as a percentage. The more extensive the corrosion, the greater the area of rust staining.

Table 5.6 shows the data for the corrosion tests. The columns entitled ‘% Area Marked’ give figures for the proportion of the filter paper test area marked by rust. Fig. 5.8 shows the rust patterns on the test areas of the tests for the 1000L experiment (the treatment times, in hours, are shown beneath each test).

Table 5.6: Corrosion Test Data for MWF Samples

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Time (hrs.)</th>
<th>% Area Marked</th>
<th>Expt.</th>
<th>Time (hrs.)</th>
<th>% Area Marked</th>
</tr>
</thead>
<tbody>
<tr>
<td>200L</td>
<td>0</td>
<td>44.2</td>
<td>1000L</td>
<td>0</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>21.0</td>
<td></td>
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<td></td>
<td>192</td>
<td>19.6</td>
<td></td>
<td>192</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>215</td>
<td>11.8</td>
<td></td>
<td>215</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>239</td>
<td>15.5</td>
<td></td>
<td>239</td>
<td>2.9</td>
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</table>

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5.3: DISCUSSION

5.3.1: Periodic Irradiation of Infected MWF

The pattern of change in total microbial count shown by the periodically-irradiated metalworking fluid in Fig. 5.1 was distinctive: 'spiked' short-term reductions, after which the microbial population of the emulsion soon returned to its original level. This pattern is similar to that of a periodically-irradiated suspension of wastewater bacteria in the work of Mechsner et al. (1991), and to that produced by the periodic addition of biocide to a metalworking fluid (Leder and Russo, 1989). Similar results would be expected in any situation where UV irradiation was not continuous,
except where the average dose applied was great enough to completely sterilise the treated liquid.

Although only short term reductions in viable counts were obtained, this preliminary experiment did show that it was possible to treat contaminated MWFs with UV in a bell photoreactor. Previous attempts to irradiate MWFs using conventional UV contactors have yielded disappointing results (P. Carpenter, personal communication). The results also suggested that in order to obtain a sustainable decrease in viable cells, a higher UV dose would be necessary. This could either be achieved by increasing the applied UV intensity (i.e. a more powerful lamp, or a greater number of lamps), or by increasing the dose rate at which the fluid was treated (e.g. by irradiating continuously).

5.3.2: Continuous Treatment Experiments

The fractional survival figures for all three experiments indicate the effectiveness of the bell contactor in reducing microbial numbers, and suppressing regrowth. The ultimate treatment capacity of the pilot-scale apparatus was never established; it might have proved possible to effectively treat still greater volumes of liquid. Also, it would be interesting to investigate the ability of the contactor to prevent the development of a significant microbial population in a freshly-prepared (i.e. uninfected) batch of emulsion.

The decline in pH noted for the infected MWF is usual in such systems, and is often taken in industry as a sign that biocidal treatment is necessary (Rossmore, 1995). This pH drop has been attributed to the oxidation of hydrocarbons by aerobic bacteria, with the concomitant production of long chain organic acids (Zuidema, 1946; King and McKenzie, 1977). In this work the rate of pH decline seemed to be unaffected by the reduction of the
microbial count of the emulsion. Freshly-mixed 4% Hocut B200 has a pH of approximately 9.0, but disinfection experiments were not started until a microbial population in the region of $10^4$-$10^7$ CFU/ml had developed. It may be that the acidification observed was caused purely by the action of free-living microorganisms in the emulsion, alternatively part at least of the effect may have been caused by adherent bacteria and fungi living on surfaces not exposed to the UV sources in the contactor. However, no evidence of obvious biofilm production was observed. A third alternative is that the pH drop may have been induced by the action of UV on the test liquid itself. If further investigations revealed one or more constituents of the MWF were susceptible in this way, it might be possible to formulate MWFs with more UV-resistant components.

The increase in temperature will have affected cell growth and metabolism in the oil emulsion. No temperature control was attempted, in order to more closely simulate the conditions which would prevail in industry. In any event, the maintenance of a constant and relatively low temperature would improve the efficiency of UV disinfection, as both growth rates and repair processes would be adversely affected in the majority of organisms.

A refinement of the isolation and identification procedure would involve the isolation of every colony from a representative plate. Replica plating (Lederberg and Lederberg, 1952) could be used to conduct some tests en masse (e.g. lactose fermentation, pyocyanin production, lecithinase activity and sorbitol assimilation). Studies in which attempts are made to monitor the fates of individual members of large and diverse microbial consortia are notoriously difficult to execute (Taber, 1976). In the 1000L irradiation experiment some 7 demonstrably different microorganisms were identified. There would certainly have been others species or strains present which the specific isolation procedures employed here would have failed to detect. The situation was further complicated because many of the species detected were
closely related and differentiation would ultimately have required the use of DNA-based methods of strain characterisation. The complete characterisation of the microflora would thus have required an enormous input of resources and would therefore have been unjustified. Notwithstanding these difficulties, some success was achieved in establishing the fates of a limited number of bacterial species.

As noted in previous work (King and McKenzie, 1977; Sondossi et al., 1985b; Passman, 1988; Mattsby-Baltzer et al., 1989; Rossmore, 1993), pseudomonads and closely-related species were by far the commonest microbial type encountered. In all these studies, the single commonest species was *Ps. aeruginosa*. *Ps. fluorescens* numbers showed a marked tendency towards decline to ultimately low numbers during all three experiments. This decline may have been due to factors other than UV light. *Ps. fluorescens* was not observed at pH values less than 5.7 during continuous irradiation. Although *Ps. fluorescens* was not isolated during the latter stages of the experiments, and was clearly strongly suppressed, it is possible that it survived at very low levels, having recolonised the system after the first and second experiments. An alternative explanation is that *Ps. fluorescens* was a common environmental contaminant. Gram positive bacteria, mainly coccoid types or tetrad formers (possibly *Micrococcus spp.*), were present only intermittently, and in low numbers. Fungi were not seen to any extent, although a yeast was isolated once, and moulds (perhaps released from biofilms) slightly more often.

*Ps. aeruginosa* appeared to be the most UV-resistant bacterium, being the only species regularly isolated toward the end of both the 400 and 1000 L experiments. In a system where there is an organism which is readily identifiable as the most resistant to UV light it would be useful to use its inactivation characteristics for 'worst case' modelling.
The results of microscopic inspection of the growth forms of microorganisms in the infected emulsion showed both bacteria and oil droplets in the samples. The bacteria observed were overwhelmingly motile rods. No aggregates of cells were seen in any field of view from any sample. The oil droplets were small (perhaps 2-3 µm in diameter). Although it was not possible to see inside these droplets, it seems unlikely that they could harbour any large unseen bacterial populations (the length of the rods themselves being not much less than the diameter of the oil droplets). Although a few dicoccoid and tetrads-forming organisms were isolated during the three continuous pilot-scale experiments (see Tables 5.1-5.3), they formed only a tiny fraction of the population. It can therefore be concluded that clumping of cells in the test liquid was negligible, and need not be taken into account in modelling UV inactivation.

Reference was made in Chapter 2 to the DNA repair mechanisms which virtually all micro-organisms possess. Samples of MWF taken during the course of the large scale experiments and maintained either in the dark or in the light, failed to reveal incontrovertible evidence that cellular repair was taking place after any appreciable time following irradiation. This does not rule out the likelihood that at least some irradiated cells were capable of some effectively ‘instantaneous’ repair. In ideal experimental conditions, many microorganisms are capable of increasing levels of repair (as measured by an increase in microbial counts) when held at low temperatures after UV irradiation. Chan and Killick (1995) investigated the recovery of suspensions of UV-treated E. coli in saline environments, however, and found that increasing salinity resulted in a diminution in the ability of sub-lethally damaged cells to repair themselves. It seems possible, therefore, that whilst the mixed microbial population colonising the MWF possessed the ability to grow and carry out maintenance-related metabolism, some environmental factor(s) may have limited its repair ability.
The recovery of the mixed microbial population once irradiation with UV had ceased, was similar to the phenomenon noted during the periodic irradiation of *Ps. stutzeri* in the prototype contactor (Figure 5.1). The noted absence of obvious post-illumination cell repair processes, as discussed above, suggests, therefore, that the recovery observed was as a result of the growth of survivors.

Prolonged irradiation did result in some fouling of the lamps, with some lamps showing more pronounced fouling than others. The most likely mechanism by which this fouling occurred was that droplets of oil-water emulsion were created by the impact of the bell on the free liquid surface and that some of these became deposited on the quartz thimbles. The heat emitted by the lamps resulted in the evaporation of water and the partial oxidation of the organic phase to form a film. This tendency towards fouling might be reduced, or even eliminated, by introducing a 'shield' which would prevent droplet deposition on the quartz thimbles. The design of the shield would have to be such that it would not itself result in UV intensity diminution. Alternatively, treatment could be interrupted after a pre-determined time to allow for cleaning of the quartz thimbles. In any commercial development of the pilot plant contactor, the cleaning process could be automated.

Table 5.6 and Fig. 5.9 show that the results from the standard corrosion test, although variable, showed no detectable trend in the ability of the emulsion to prevent the corrosion of the wet cast iron chips. The extent of corrosion was greater than normally seen with similar products. Freshly-mixed 4% Hocut B200 produced a corrosion area result of 15.0%, whereas other formulations generally produce negligible (i.e. less than 1%) corrosion at similar concentrations when fresh. The value for percentage of test area covered by rust corresponding to a ‘failure’ (i.e. MWF no longer able to perform its rôle properly) is 5%. The high level of corrosion may be due to the fact that Hocut B200 is meant to be used as part of a binary system with a biostable additive
(Hocut B205), which was omitted in order to facilitate the initial infection of the test emulsion. Some of the variation in the results is due to the image processing area estimation method used (conventionally the amount of corrosion on a test region is determined by counting rust-stained squares on a clear grid placed over the test paper). The test for the 239 hour sample from the 1000 L experiment does not appear significantly different from that for 215 hours, but the percentage of test area stained by corrosion was recorded as 2.9 and 14.3% respectively. It seems that the computer software was too sensitive to differences in the colour intensity of the rust staining, but it does represent the basis of a novel approach to obtaining non-observer biased quantitative data from standard corrosion tests.

5.4: CONCLUSIONS

The bell contactor was effective in reducing the microbial loading of badly infected soluble oil emulsions to stable low levels. Indeed, the treatment reduced microbial counts to near sterility in the 400 and 1000 L experiments (the whole of the latter passing the UV sources only once every 50 minutes). The levels of microbial contamination ($10^4$-$10^7$ CFU/ml) prior to each experiment were equivalent to those seen in industry for a typical case of serious infection (Mattsby-Baltzer et al., 1989). Such a reduction should reduce any biological infection risk to metal workers, and may extend the life of the emulsion itself.

Although very low numbers of dicoccoid and tetrad-forming bacteria were observed, it appears that the vast majority of the free-living bacteria present in the cutting oil were single cells rather than in cell aggregates or 'clumps'. Further, it appears that microorganisms inhabited the water rather than oil phase of the emulsion.
Isolation and identification of the microorganisms present in the MWF showed the majority (both in species and numbers) of them to pseudomonads. This is in line with the findings of other authors (King and McKenzie, 1977; Mattsby-Baltzer et al., 1989; Rossmore, 1993). *Ps. aeruginosa* was the commonest organism; for both the 400 and 1000 L experiments.

Attempts were made to monitor the fates of individual bacterial species present in the MWF during UV treatment. Some success was achieved in so far as it was revealed that certain species, notably *Ps. aeruginosa*, seemed to persist throughout the entire period of treatment, whereas others e.g. *Sphingomonas paucimobilis*, were only detected over relatively short periods.

The mixed microbial populations present in the MWF were unable to demonstrably conduct either light or dark repair under the environmental conditions prevailing in the MWF. However, regrowth of elements of the population occurred once UV irradiation had ceased.

Evidence was obtained to show that fouling of the quartz thimbles shrouding the UV lamps occurred. The fouling was more pronounced on the external lamps.

There was no detectable variation in the corrosion inhibition capability of the MWF investigated over the period of the disinfection experiments, but an exciting method of quantitatively evaluating corrosion was developed.
CHAPTER 6: MATHEMATICAL ESTIMATION OF UV DOSE

6.1: INTRODUCTION

The difficulties in obtaining consistent estimates of UV doses by actinometry and bioassays were discussed at some length in Chapter 4. One method of circumventing these difficulties would be to devise a mathematical model which would enable UV dose to be predicted. A number of mathematical models exist (Lance and Perry, 1953; Hovingh, 1977; Hoffman et al., 1980 and Ramos, 1988) which enable estimates to be made of the profiles of liquid bells. Data can also be derived from these models for the thickness, velocity and spatial orientation of the liquid films comprising the bell. In this chapter a description is given of the methods by which the bell model first proposed by Lance and Perry (1953) was used in association with techniques for predicting the light intensity in the vicinity of sources. This enabled estimates to be made of the UV doses delivered to bells by various arrangements of sources.

As in all similar circumstances, confidence in the predictions produced by mathematical models can only be justified if the predictions can be verified. In this case, verification of dose predictions from the devised model were made with reference to the dose measurements presented in Chapter 4.
6.2: MODELLING BELL SHAPE

The majority of liquid bell models have employed tangential-normal co-ordinate systems, following the lead of the first mathematical treatment attempted by Boussinesq (1869a, b). As mentioned in 2.5.1, one of the more recent and most widely quoted models of this type is that proposed by Lance and Perry (1953), and it was decided to employ their model here. This states that the equation for the radius of the bell is given by the following second order differential equation.

\[
1 + z'^2 \left\{ \frac{Y}{e} + \beta z' + \alpha r(1 + 2'2) \right\} = z''(e - \beta r)
\] (6-1)

The full derivation of this expression (and the definition of all terms used) is given in Appendix E.1.

This equation can not be solved analytically, and solutions to it were obtained by employing a method based on Euler's polygon method for first order differential equations (see Appendix E.1 also).

6.3: LIGHT INTENSITY MODELLING

There are two types of light intensity model: incidence models and emission models. Incidence models are built up using measured values for light incident at a number of points, whereas emission models can predict emission from lamps at any given point. Generally, emission models are used for light intensity modelling, since they allow the modelling of intensity fields at the design stage, rather than after reactor construction. In light field models which are of the emission type, the light source is assumed to comprise a series of ‘point sources’. Light field models are built up by dividing the relevant region into a series of elements, and determining the ‘point intensity’
values for all such element from every point source. In the case of a model for a liquid UV contactor, this will be the liquid irradiated in the contactor. It is also necessary to take account of the orientation of the surface of a liquid element to any point source and also of the path length of light through the treated liquid. Emission models may be sub-divided into two groups: line source (LS) models, and extense source (ES) models.

Line source models have been favoured for the analysis of photoreactors as they generally allow a simple numerical treatment, and an analytical solution can sometimes be reached. This type of model is built up by the summation of theoretical point light sources along the axis of the lamp. Individual point sources are assumed to emit light in different ways by different kinds of line source model (Harris and Dranoff, 1965; Jacob and Dranoff, 1970; Akehata and Shirai, 1972).

Methods which take into account the three-dimensional shape of real light sources are known as extense source models. Extense source models are of two general types. Models in which the source is considered to consist of emitters evenly distributed over the lamp surface (superficial emission, ESSE models) are used for coated lamps (e.g. domestic strip lights). Volumetric emission (ESVE) methods, consider sources to be made up of emitters uniformly distributed within their volume. ESVE models are the most suitable for the analysis of germicidal UV lamps (which are uncoated).

6.3.1: Light Intensity Modelling for the Bell Contactors

The UV sources used in this work were cylindrical. In modelling them it was decided to assume that they emitted UV light evenly throughout their volumes, i.e. that they exhibited volumetric emission. This ESVE-type treatment of the UV sources assumes that each point source emits light in all directions. Each cylindrical UV source was divided into regions of equal
volume. Further, each volume element was assumed to have a UV point source located at its centre.

**Fig. 6.1: Division of UV Source**

![Diagram of UV source division](image)

\[ NC = \text{the number of circles dividing the source cross-section vertically (2 in this case).} \]

\[ NL = \text{the number of lines dividing the source cross-section vertically (2 in this case).} \]

\[ NZ = \text{the number of zones into which the source is divided along its length (8 in this case).} \]

Fig. 6.1 shows how a UV source is divided along its length into a number of zones, and how each zone is divided vertically by lines and circles such that the source is notionally divided into regions of equal volume. Dividing the source in this manner ensures that the lamp output is treated as being symmetrical.

Using the terminology of Jacob and Dranoff (1970), the UV intensity distribution from a point source in an arc lamp can be described as,

\[
I = \frac{S}{4\pi \rho^2} e^{-\mu \rho} \quad (6-2)
\]
where $I =$ the intensity of UV light of wavelength 253.7 nm, $S =$ the power output of the point source (253.7 nm), $\rho =$ distance between the point source and the receptor location, and $\mu =$ absorption coefficient of the medium at 253.7 nm.

When the medium is air and the distances between source and receptor are relatively small it can safely be assumed $\mu = 0$.

6.4: ESTIMATING UV DOSES DELIVERED TO BELLS

UV dose estimation required the coupling together of the Lance and Perry model for bell profile estimation with the light intensity model. In order to achieve this it was necessary to divide the bell into a number of elements small enough so that UV intensity can be taken to be constant within each of them. The sources were notionally divided into areas of equal volume, each containing a UV point source of equal power output. It was then necessary to calculate the point intensity of UV from each point source at a central point at which light strikes the surface of each bell element. A correction was applied to account for the absorption of UV which occurred within each bell element. Finally, by summing the intensities for each bell element it was possible to arrive at the average UV intensity at which the entire bell was irradiated.

A computer program was written in Turbo Basic (Borland International, Scotts Valley, CA, U.S.A.) which was capable of calculating both the parameters relating to the liquid bell, and the average dose and intensity values for all lamp configurations employed. A copy of the program (belldose.bas) and a full listing terms used (with notes) are to be found in Appendices E.5 and E.6 respectively.
6.4.1: Case I: Single Centrally-Mounted UV Source

The situation in which the liquid bell was only irradiated by the single centrally-mounted source is the simplest case possible. This applied to the laboratory-scale apparatus and when desired, to the pilot-scale apparatus. The central position of the source within the bell means that the system is radially symmetrical. As a consequence of the use of the Lance and Perry numerical solution for the bell, it is already divided into sections of equal length along its profile. There is no need to divide the bell circumferentially since, due to the symmetry mentioned above, the UV intensity will be the same at any given radial distance.

6.4.1.1: View Factors for Liquid Bell Elements

The orientation of a liquid surface to a UV point source will affect the average UV intensity at that surface (see Fig. 6.2).

**Fig. 6.2: The View Factor for a Bell Element**

For the first case in Fig. 6.2, (a), where the incident UV light (intensity = $I_0$) meets the surface of the bell element normally the intensity at the surface is given by $I_0$, but in the general case where the surface is inclined relative to the source, then
The term $\cos \lambda$ is called the 'view factor'. The view factor changes for each point source/bell element pairing. The full method for the calculation of the view factor is given in Appendix E.2.

6.4.1.2: Calculating the UV Path Length Through a Bell Element

Using the Lance and Perry solution for the profile of the liquid bell, it is relatively straightforward to calculate the thickness of the liquid sheet, $h$, at any point by continuity. This value will be equivalent to the path length of light through a bell element only when the light path is perpendicular to the surface of the bell element, however. Fig. 6.3 illustrates how the light path length is greater than the thickness of the element for any other point source/bell element orientation.

**Fig. 6.3: UV Path Length Through Bell**

It follows that:

$$h' = \frac{h}{\cos \lambda}$$  \hspace{1cm} (6-4)
where \( h \) = element thickness, \( h' \) = UV path length, and \( \cos \lambda \) = view factor.

6.4.1.3: Calculation of the Average UV Intensity in the Bell Element

Equations (6-2), (6-3), can be combined with the expression for the intensity reduction due to absorption

\[
\left( \frac{I}{I_0} \right) = \frac{1}{N_T} \sum_{k=1}^{N_T} e^{-\mu_p}
\]

(6-5)

(where \( N_T \) = the number of points along the path length at which intensity is calculated)

to yield the following equation for the average UV intensity in a particular bell element:

\[
\bar{I} = \sum_{j=1}^{N_T} \frac{S_p}{4\pi \rho_j^2} \cdot \cos \lambda_j \cdot \left( \frac{\bar{I}_j}{(I_0)_j} \right)
\]

\\( \Rightarrow \)

\[
\bar{I} = \frac{S_p}{4\pi} \sum_{j=1}^{N_T} \frac{1}{\rho_j^2} \cdot \cos \lambda_j \cdot \left( \frac{\bar{I}_j}{(I_0)_j} \right)
\]

(6-6)

where \( N_p \) = the number of UV point sources, \( S \) = the total power output of the UV source and \( S_p = S/N_p \) = power output of one theoretical point source. Selecting excessively high values of \( N_p \) resulted in increased computation time for relatively minor gains in the accuracy of the solutions obtained. A value of \( N_p \) of 60 provided an acceptable compromise.
6.4.1.4: Calculation of the Average Dose Per Pass for the Whole Bell

In UV dose modelling an average dose (dose distribution) is produced from an intensity field distribution, and the retention time, e.g.:

\[
\text{UV Dose} = \text{Intensity} \times \text{Time}
\]

By means of equation (6-6) the average UV intensity along the whole bell profile can be calculated. The average residence time of a 'particle' of liquid in a bell element may be expressed as:

\[
\bar{t} = \frac{A_l}{v}
\]  

(6-7)

where \(\bar{t}\) = residence time, \(A_l\) = arc length (a fixed variable to be set in the computer program), and \(v\) = velocity of the bell element (calculated by the numerical solution to the bell profile).

If there are \(C\) points calculated along the bell profile, there will be \((C-1)\) bell elements. Therefore:

\[
\overline{D}_B = \sum_{j=1}^{C-1} \bar{D}_j \cdot \bar{t}_j
\]  

(6-8)

where \(\overline{D}_B\) = the average UV dose that a particle of liquid is exposed to in one pass through the contactor.

Finally,

\[
\bar{t}_B = \sum_{j=1}^{C-1} \bar{t}_j
\]  

(6-9)
and

$$\bar{I}_b = \frac{\bar{D}_b}{t_b}$$  \hspace{1cm} (6-10)

where $\bar{t}_b$ = the average residence time in the whole bell, and $\bar{I}_b$ = the average UV intensity in the whole bell.

6.4.2: Case II: Irradiation by Multiple UV Sources

Where the pilot-scale apparatus was used, it was possible to irradiate the liquid bell produced in several different ways. Where irradiation was conducted solely with the single central UV source, the solution was as described above. Two additional irradiation regimes were also possible:

(i) Using the sixteen external circumferentially-mounted UV sources, i.e. external multilamp irradiation.
(ii) Using all available UV sources simultaneously, i.e. multilamp irradiation from both sides.

In both of these cases a slightly different modelling approach to that used above in 6.4.1 must be employed since the radial symmetry involved in irradiation with a single central source does not apply in the same manner. Consequently it becomes necessary to divide the liquid bell radially, as well as the division along the bell profile imposed as part of the solution of the equation for the bell’s surface. Each sector along the bell profile is further divided into 360 one-degree sectors. It should be noted that different bell radius values may lead to sectors of the bell being either unirradiated (Fig. 6.4a) or irradiated by more than one fixed external UV source (Fig. 6.4b). Further, these differences pertain not only to different liquid bells, but at
different values of the $z$ co-ordinate (i.e. vertical distance from the nozzle) within the same bell.

**Fig. 6.4: Effect of Varying Bell Radius on Irradiation by Fixed External Sources**

![Diagram of bell profile and irradiation](image)

In order to correctly model multi-lamp bell irradiation, it is necessary to know how much of the bell is irradiated by any one external source at any particular value of $z$. The determination of this 'critical angle' is given in Appendix E.3. In none of the cases modelled was the radius of the bell surface such that portions of the bell remained unirradiated, rather, in all cases the effects of overlap had to be considered. The method chosen to do this is also described in Appendix E.3.

### 6.4.2.1: View Factor Angle and UV Path Length

Both the view factor angle and the UV path length are calculated in a manner similar to that used for the centrally-mounted lamp, although attention must be paid to the different orientation of the lamp to the liquid. The amount of computing required is also considerably greater, since both parameters must be calculated in three dimensions. The methods employed to achieve this are given in full in Appendix E.4.
6.5: RESULTS

Fig. 6.5 shows the three dimensional outlines of two bells generated using the Lance and Perry (1953) model superimposed over photographs of bells. The predicted bell outlines were obtained using identical parameters (i.e. flow rate, gap opening etc.) to those employed in producing the actual bells. The degree of agreement between the modelled shapes and photographs is very good.

Using commercially available software (AutoCAD 12, Autodesk Inc., San Rafael, CA, U.S.A.), bell profiles generated by the model could be rotated to produce three dimensional-type representations which may be viewed from different angles. Three different views of a bell obtained by this method are presented in Fig. 6.6.

Fig. 6.7 (a and b) illustrates the effect of changing nozzle gap opening on bell profile as predicted by the model. At low gap openings the liquid velocity is relatively high as it leaves the nozzle and the bells formed assume a broad shape. As the gap opening decreases, the bell profile narrows until it converges towards its longitudinal axis.

Figs. 6.8 and 6.9 show modelled values of film thickness and liquid velocity plotted with experimental values obtained from high speed ciné film of actual water bells obtained by Shama et al. (1996). Tiny air bubbles entrained in the bell sheet were visible under certain conditions of illumination and enabled the liquid velocity to be measured directly. The continuity equation made it possible to derive thickness values from measured velocity values.
Fig. 6.6: Different Views of a Liquid Bell Generated by AutoCAD From Data Generated Using the Lance and Perry (1953) Model
FIG. 6-7a: The Effect of Changing the Nozzle Gap Opening on Bell Profile ($Q = 2.5E.4 m^3/s$)
FIG. 6.7(b): The Effect of Change in Nozzle Gap Opening on Bell Profile (Q = 2.5E-4 m$^3$s$^{-1}$)
Fig. 6.8: Comparison of Modelled Film Thickness with Experimentally Derived Values
('Sequence' refers to film sequences of the tracks of entrained air bubbles seen in water bell, see Shama et al., 1996)

![Graph showing comparison of modelled film thickness with experimentally derived values.]

Fig. 6.9: Comparison of Modelled Liquid Velocity with Experimentally Measured Values
('Sequence' refers to film sequences of the tracks of entrained air bubbles seen in water bell, see Shama et al., 1996)

![Graph showing comparison of modelled liquid velocity with experimentally measured values.]

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Tables 6.1 and 6.2 show predicted UV doses for both the laboratory and the pilot-scale apparatus. A range of flow rates were modelled for the nozzles of both sets of apparatus, some of which corresponded to ‘real’ bells for which experimental data were available (see Tables 6.3-6.6).

Figs. 6.10 and 6.11 show the effects of changing nozzle gap on average dose and average (median) thickness respectively. The data was generated for the given liquid flow rates for the laboratory-scale apparatus (i.e. central lamp only case), with an internal pressure of +2 Pa. Shama and Rice (unpublished) measured the internal pressure of water bells of approximately similar dimensions to those generated here and found values in the region of +4.5 Pa. At each flow rate, reducing the nozzle gap below the values shown would result in artefactual solutions in which the liquid film intersects itself (at an internal pressure value of +2 Pa); this ‘spiralling’ phenomenon was noted by Lance and Perry. Increasing the nozzle gap beyond the values shown leads to the convergence of the bell film into a jet before the end of the contactor is reached.

Figs. 6.12-6.15 show the effects of varying nozzle gap on average dose (for the central lamp, the 16 external lamps, and for all lamps lit) and thickness for the pilot-scale apparatus. For each curve, the considerations detailed above applied, except that the bells were modelled with no internal overpressure. Additionally, care was taken that modelled bells did not have maximum radii greater than 17.25 cm (the radial distance of the external lamps from the central axis of the bell). The majority of the curves obtained were monotonic, with the exception of the curves obtained at a flow rate of 1300 L/hr. for the pilot-scale apparatus (Figs. 6.12 and 6.14). This anomaly is commented on further in the Discussion.
Table 6.1: Predicted Dose Values for the Laboratory-Scale Apparatus

<table>
<thead>
<tr>
<th>Flow Rate (L/hr)</th>
<th>Nozzle Gap (mm)</th>
<th>Ave. Dose (mW-s/cm²)</th>
<th>Ave. Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>3.0</td>
<td>2.02</td>
<td>0.58</td>
</tr>
<tr>
<td>650</td>
<td>1.9</td>
<td>0.50</td>
<td>0.28</td>
</tr>
<tr>
<td>700</td>
<td>3.0</td>
<td>0.67</td>
<td>0.42</td>
</tr>
<tr>
<td>800</td>
<td>3.0</td>
<td>0.49</td>
<td>0.38</td>
</tr>
<tr>
<td>860</td>
<td>4.6</td>
<td>1.01</td>
<td>0.66</td>
</tr>
<tr>
<td>900</td>
<td>3.0</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>1000</td>
<td>3.0</td>
<td>0.35</td>
<td>0.34</td>
</tr>
</tbody>
</table>

(Lamp power output at 253.7 nm calculated as 6.1 W, method as per 3.5. • Estimated by bell model.)

Table 6.2: Predicted Dose Values for the Pilot-Scale Apparatus

<table>
<thead>
<tr>
<th>Flow Rate (L/hr)</th>
<th>Nozzle Gap (mm)</th>
<th>Ave. Dose (mW-s/cm²)</th>
<th>Ave. Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single Lamp</td>
<td>External Lamps</td>
</tr>
<tr>
<td>990</td>
<td>1.4</td>
<td>0.84-1.10</td>
<td>2.30-3.02</td>
</tr>
<tr>
<td>1080</td>
<td>2.6</td>
<td>2.81-3.69</td>
<td>2.10-2.75</td>
</tr>
<tr>
<td>1100</td>
<td>2.5</td>
<td>1.77-2.32</td>
<td>2.33-3.06</td>
</tr>
<tr>
<td>1200</td>
<td>2.5</td>
<td>1.30-1.70</td>
<td>2.38-3.12</td>
</tr>
<tr>
<td>1300</td>
<td>2.5</td>
<td>1.11-1.46</td>
<td>2.48-3.25</td>
</tr>
<tr>
<td>1400</td>
<td>2.5</td>
<td>0.97-1.27</td>
<td>2.59-3.40</td>
</tr>
<tr>
<td>1500</td>
<td>2.5</td>
<td>0.89-1.16</td>
<td>2.77-3.64</td>
</tr>
</tbody>
</table>

(• range of predictions due to the differing values for the power output at 253.7 nm of the lamps used, i.e. maker’s figure, 13.8 W, and the figure calculated, 18.1 W, method as per 3.5. • Estimated by bell model.)
Fig. 6.10: Effect of Changing Nozzle Gap on Predicted Average UV Dose (Laboratory-Scale Apparatus)

Fig. 6.11: Effect of Changing Nozzle Gap on Predicted Average Film Thickness (Laboratory-Scale Apparatus)
Fig. 6.12: Effect of Changing Nozzle Gap on Predicted Average UV Dose (Pilot-Scale Apparatus- Central Lamp Only)

Fig. 6.13: Effect of Changing Nozzle Gap on Predicted Average UV Dose (Pilot-Scale Apparatus- External Lamps Only)
Fig. 6.14: Effect of Changing Nozzle Gap on Predicted Average UV Dose (Pilot-Scale Apparatus - All Lamps)

![Graph showing the effect of nozzle gap on predicted average UV dose.](image)

Fig. 6.15: Effect of Changing Nozzle Gap on Predicted Average Film Thickness (Laboratory-Scale Apparatus)

![Graph showing the effect of nozzle gap on predicted average film thickness.](image)
6.6: DISCUSSION

The Lance and Perry model was revealed as producing realistic estimates of bell parameters by comparison with experimentally derived measurements such as film thickness and velocity (Figs. 6.8 and 6.9, respectively). In addition, overall morphological agreement was obtained with bells generated using the model and with photographs of bells (Fig. 6.5). This was despite the fact that the actual bells generated using the nozzle were probably subject to phenomena not accounted for in the Lance and Perry model such as air drag and effects similar to the capillary instability seen in simple jets (Rayleigh, 1896; Meyer and Weihs, 1987). Alternative models (Hovingh, 1977; Hoffman et al., 1980; Ramos, 1988) with potential applications to liquid bells have been developed over recent years. However, they do not appear to offer any obvious advantages over that of Lance and Perry and so were not evaluated here.

Some workers have employed cylindrical co-ordinate systems to model liquid curtains (Hovingh, 1977; Hoffman et al., 1980; Ramos, 1988). Equations (6-11) and (6-12) below are those which Ramos has developed for the radial and vertical equations of motion from the tangential-normal systems previously employed:

\[
\rho b \frac{d^2 R}{dt^2} = -(f_i + f_x) \sin \theta - \cos \theta \left[ \frac{2 \sigma \cos \theta}{R} + \frac{2 \sigma}{r_v} + p_e - p_i \right] \quad (6-11)
\]

\[
\rho b \frac{d^2 z}{dt^2} = \rho gb - (f_i + f_x) \cos \theta + \sin \theta \left[ \frac{2 \sigma \cos \theta}{R} + \frac{2 \sigma}{r_v} + p_e - p_i \right] \quad (6-12)
\]

where \( t \) is time, \( z \) is the axial location of an element of the liquid curtain, and \( r_v \) is given by
\[
\frac{1}{r} = -\frac{d^2R}{dz^2}/\left[1 + \left(\frac{dR}{dz}\right)^2\right]^{1/2}
\]  

(6-13)

In contrast to the work of Lance and Perry these later workers (Hoffman et al., 1980; Ramos, 1988) have modelled liquid curtain shape and convergence length as functions of dimensionless groups such Fr (the Froude number) and N (the convergence number, \(N = We/Fr^2\), where \(We\) is the Weber number). Whilst such systems employing cylindrical co-ordinates are convenient for liquid curtains produced by vertical annular nozzles, they do not appear to offer any advantage over the tangential-normal systems previously employed for liquid bell modelling.

It can be seen by inspection of Figs. 6.11 and 6.15 that, for any particular flow rate, average film thickness increases with increasing nozzle gap. As the initial velocity (i.e. \(v_y\), the component of velocity perpendicular to gravity) decreases with increasing nozzle gap, the maximum radius of the bell produced also decreases (see Fig. 6.7). One would therefore expect the average dose from the central lamp to increase with increasing nozzle gap, and the converse to be true for that from the external lamps, because of the differences in the distance of the liquid film from the irradiating sources. Inspection of the predicted data for the single central source case (Figs. 6.10 and 6.12), and the external sources irradiation only case (Fig. 6.13) reveals that this is indeed the case. The only slight anomaly is the low dose predicted for the 1300 L/hr. flow rate, with a nozzle gap of 3.5 mm, irradiated only by a centrally-positioned source, which seems to coincide with a relatively large average sheet thickness. Fig. 6.14 shows the data for irradiation by all 17 sources. Even though the dose contribution from the external sources is in all cases greater than that from the central source, the curves in Fig. 6.14 are generally similar in shape to those Fig. 6.12 (i.e. those for central lamp only irradiation); this occurs because there is greater variation (at any flow rate) in
dose from one nozzle gap setting to another for central lamp irradiation than for external lamp irradiation.

Selected values of predicted UV doses (from Tables 6.1 and 6.2) corresponding to flow rates for which actual measurements of dose were made are presented in Tables 6.3-6.6. The data of Table 6.3 is for the single lamp laboratory contactor and that of Tables 6.4-6.6 are for different irradiation regimes achieved with the pilot-scale (multi-lamp) unit.

Examination of Table 6.3 shows that UV doses estimated by the *E. coli* bioassay, actinometry and on the basis of direct radiometer readings show good agreement with the doses predicted by the model but are all lower than the latter. The doses estimated using the *Ps. stutzeri* bioassay stand out as being relatively high. Moreover, they suggest that dose decreases with increasing flow rate, contrary to the predictions obtained using the model. The *Ps. stutzeri* bioassay was previously criticised in Chapter 4 for lacking sensitivity and capable of yielding inaccurate doses because of the presence of a shoulder on the dose response curve of the organism (Fig. 4.1). In the region of the shoulder relatively small changes in fractional survival result in relatively large changes in estimated dose. The radiometer-based dose estimates were obtained using point intensity measurements and retention times (since the retention times for the contactor are very similar for different flow rates, these estimates are relatively insensitive to liquid flow rate).

Operation at the two flow rates shown in Table 6.3 was achieved at different nozzle gap openings (see Tables 6.1 and 6.2). This parameter is an important determinant of bell characteristics. The nozzle gap opening determines the lateral velocity at which the liquid leaves the nozzle, this will in turn influence both the maximum radius which the bell can assume and also the thickness of the liquid film. Large gap openings will result in the formation of bells of relatively low liquid velocity, large film thickness values and small maximum
diameter. For the case of irradiation of the bell by a single central lamp, these conditions imply that the liquid sheet comprising the bell will remain relatively close to the UV lamp and will as a consequence receive a higher dose than bells of larger maximum diameters.

At 650 L/hr. the velocity of the liquid issuing from the nozzle gap would be 0.71 m/s and the maximum diameter of the resulting bell would be 9.60 cm; the film thickness at this position would be 0.15 mm. At 850 L/hr. the liquid velocity is 0.39 m/s and the maximum bell diameter is 5.08 cm, with film thickness at this diameter of 0.56 mm. At the lower flow rate the liquid sheet is relatively distant from the UV lamp, whilst at the higher flow rate the distance of the bell from the UV lamp is less, which implies a higher UV dose in the latter than in the former case, as predicted by the model. The retention times for the 650 and the 860 L/hr. bells were practically the same (both 0.22 s, to 2 d.p.), although where one bell is of a much greater diameter than another (i.e. the length along its profile is much greater) the retention time will be slightly greater for the same distance fallen.

Closer agreement between model predictions and actinometric dose estimates is evident for single lamp irradiation using the multi-lamp contactor (Table 6.4). This pattern is repeated for the case of bell irradiation by the external lamps only (Table 6.5), but as previously the Ps. stutzeri appear high. In the case of irradiation by all sources (Table 6.6) the actinometric dose estimates are notably lower than the model predictions which now appear consistent with the Ps. stutzeri derived estimates.

This apparent contradiction with the comments made above regarding this bioassay, can be explained by the fact that the doses of Table 6.6 are larger than those of Tables 6.4 and 6.5 and no longer fall in the shoulder region of the dose-response curve but are now in the linear region where dose estimates can be made with improved accuracy.
There is also best agreement between predicted dose values and actinometric measurements as to which flow rate setting will produce the higher dose (i.e. 860 L/hr bell has a higher average dose than the 650 L/hr bell, and the 1080 L/hr bell greater than the 990 L/hr bell); in one case (see Table 6.2) the bioassay dose estimates indicate the reverse to be true.

Certain other anomalies (i.e. the bell with the smallest average thickness value does not necessarily have the highest dose, the external source only model predictions [see Table 6.5] reversing the overall trend in that the 990 L/hr. bell has a higher dose estimate than the 1080 L/hr bell) may be explained by the distance from the bell to the lamp concerned. The square of the distance of any particular element in the bell from the lamp is used by the model to calculate the incident UV intensity; for the centrally-lit case then, one would expect a higher dose estimate for a bell whose profile was closest to the central lamp axis. It should be remembered, however, that when the bell is very close to the external sources, the sector irradiated by each source (and therefore the theoretical total) is reduced.

Table 6.3: Irradiation of Laboratory-Scale Apparatus

<table>
<thead>
<tr>
<th>Flow rate (L/hr.)</th>
<th>Average UV Dose (mW-s/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ps. stutzeri E. coli</td>
</tr>
<tr>
<td></td>
<td>Bioassay Bioassay</td>
</tr>
<tr>
<td></td>
<td>Actinometric Radiometer</td>
</tr>
<tr>
<td></td>
<td>Model prediction</td>
</tr>
<tr>
<td>650</td>
<td>2.34 0.12 0.27 0.43 0.50</td>
</tr>
<tr>
<td>860</td>
<td>1.41 - - 0.44 1.01</td>
</tr>
</tbody>
</table>
### Table 6.4: Irradiation of Pilot-Scale Apparatus- Central Source Only

<table>
<thead>
<tr>
<th>Flow rate (L/hr.)</th>
<th>Average UV Dose (mW-s/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. stutzeri Bioassay</td>
<td>Actinometric</td>
</tr>
<tr>
<td>990</td>
<td>1.10</td>
</tr>
<tr>
<td>1080</td>
<td>3.77</td>
</tr>
</tbody>
</table>

### Table 6.5: Irradiation of Pilot-Scale Apparatus- External Sources Only

<table>
<thead>
<tr>
<th>Flow rate (L/hr.)</th>
<th>Average UV Dose (mW-s/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. stutzeri Bioassay</td>
<td>Actinometric</td>
</tr>
<tr>
<td>990</td>
<td>-</td>
</tr>
<tr>
<td>1080</td>
<td>4.35</td>
</tr>
</tbody>
</table>

### Table 6.6: Irradiation of Pilot-Scale Apparatus- All Sources

<table>
<thead>
<tr>
<th>Flow rate (L/hr.)</th>
<th>Average UV Dose (mW-s/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. stutzeri Bioassay</td>
<td>Actinometric</td>
</tr>
<tr>
<td>990</td>
<td>1.36</td>
</tr>
<tr>
<td>1080</td>
<td>3.06</td>
</tr>
</tbody>
</table>

(± range of predictions due to the differing values for the power output at 253.7 nm of the lamps used, i.e. maker's figure, 13.8W, and the figure calculated, 18.1W, method as per 3.5)

The mathematical modelling of UV intensity, and thus dose, in UV photoreactors has been employed by many authors (Jacob and Dranoff, 1970; Sugawara et al., 1981; Esplugas et al., 1983; Alfano et al., 1986; Suidan and Severin, 1986; Blatchley et al., 1995; Braunstein et al., 1996). However, relatively few workers have presented verification of their predictions. Qualls and Johnson (1983; 1985) compared modelled light intensities with estimates obtained with a B. subtilis injection bioassay. The predicted intensity figures 'corresponded fairly well' with the assay data, although in one case the predicted values were consistently higher than the experimental figures (Qualls and Johnson, 1983). Braunstein et al. (1996) also compared experimental data from a bioassay (MS2 coliphage) with figures produced by...
a mathematical radiation model. Results showed that the predicted estimates were within 10% of the bioassay figures. At first it appears surprising that these two groups of workers, both of whom employed a simple point source summation (PSS) line source model, should achieve apparently better correlation than was provided by the more sophisticated volume emission model. It must be remembered, however, that the UV contactors modelled in both cases were of much simpler geometry than a liquid bell (simple cylinders and an open channel respectively), thus presenting far fewer difficulties in modelling.

The models at present used make use of certain simplifying assumptions. The first of these ignores reflection of UV light from the surfaces of the bell. The second assumption is that light which has once penetrated the bell sheet is assumed not to reach another surface of the hollow bell film and exert an effect at that surface (likely to be the case for highly absorbing liquids at least). If the second assumption holds true, the lamp shielding effects which have been noticed for some multilamp UV contactors (Qualls and Johnson, 1985) will not be relevant in this system (i.e. the central source will not shield any part of the bell from the light output of any of the external sources). A further assumption is that intensity at any point in the irradiated liquid was treated as the sum of the contributions from each lamp. These assumptions are similar to those of other workers (Scheible, 1987; Nieuwstad et al., 1991; Blatchley et al., 1995; Braunstein et al., 1996), but the last fails to account for the possible effects of inter-lamp UV interference postulated in Chapter 4. If this is the case, modelled dose estimates will be higher than experimental estimates for multilamp irradiation.

6.7: CONCLUSIONS

The hydrodynamic model of Lance and Perry (1953) enabled the shape, liquid velocity and film thickness of the bells generated using the annular jet nozzle
to be predicted. Estimates for these parameters obtained using this model were in close agreement with experimentally determined values.

The UV intensity field in the vicinity of UV lamps was modelled using a volumetric emission model. This was combined with the hydrodynamic bell model to yield estimates of UV doses delivered to bells. The model produced dose estimates which were in line with those produced by both bioassay and actinometric methods.

In particular, the results obtained using the model were sufficiently encouraging to enable model estimates to be used with greater confidence. This would enable, for example, operating regimes resulting in maximum UV doses to be identified and subsequently verified, resulting in a reduced programme of experimentation.
CHAPTER 7: DISINFECTION MODELLING

7.1: INTRODUCTION

The effect of UV on an individual living cell is dependent on many factors (Fig. 7.1), including for example the capacity of the cell to repair UV-induced damage and the nature of the environment in which the cell is irradiated. UV may induce mutations of various degrees of severity, or sub-lethal injury or ultimately, death. Whilst the latter is an absolute state, the distinction between extensive mutagenesis and sub-lethal injury would be almost impossible to define precisely. Environmental factors such as pH and temperature can be expected to exert an influence on the rate of growth of sub-lethally injured cells and also on the rates of enzymatic repair processes.

Fig. 7.1: Possible Fates of a UV-Exposed Cell

Viable Microbial Cell

Exposure to UV

Does the cellular DNA sustain damage?

No

Yes

Is the environment conducive to growth?

Yes

No

Are the lesions repaired?

Yes

No

Stasis

Cell divides

Cell dies

Disinfection processes will normally be targeted against media containing assemblages or populations of cells: these may be either mixed or axenic. In a mixed population one can expect a broad range of characteristics to be displayed by members of the population in regard to repair capacity as well as other characteristics. However, even in axenic populations, not all the
individuals will exhibit identical responses to UV (Jagger, 1967). This variation being due in part to the distribution of cell age normally found in populations. Predicting the performance of disinfection processes operating in circulatory (rather than single pass) mode will be further complicated as the microbial population will be subjected to sequential exposure to UV.

In this chapter attempts at predicting the disinfection performance of circulatory disinfection processes are described. In particular, the large scale irradiation experiments of MWF are analysed and attempts made to predict cell numbers as a function of time, these are subsequently compared with experimental values. In order to achieve this objective, use is made of the UV dose prediction models developed for the thin film contactor described in the previous chapter. Additional data was also required, and whilst in certain cases it was available, in other cases recourse had to be made to the best available data. As a consequence the predictions presented here should only be thought of as preliminary.

7.2 DISINFECTION MODEL

Using a series-event kinetic model (Severin et al., 1983a) applied to a flow-through completely mixed reactor, single-pass survival can be expressed as:

\[
\frac{N}{N_0} = 1 - \left[ 1 + \frac{1}{k\Gamma t} \right]^n
\]

(2-9)

where \( n \) = the threshold number of damage required for UV inactivation, \( k \) = the batch inactivation rate constant, \( \Gamma = \text{Ave UV intensity in treated liquid} \), and \( t = \text{retention time} \).
7.2.1: Disinfection Constants (k and n)

Dose response curves for the mixed microbial populations comprising the 200, 400 and 1000 L batches of MWF were not determined during the course of the irradiation experiments. However, the results of Chapter 5 (see e.g. Table 5.3) revealed that pseudomonads were prominent members of the population in all cases. Whilst species belonging to this genus can be expected to display a range of sensitivities to UV, some guidance as to realistic values of the constants to be used in the Severin model was available from the dose response curve of \textit{Ps. stutzeri} (Fig. 4.1). In the absence of data, it was assumed that the kinetic constants for this organism could be taken as representative of the mixed populations. Initial predictions were made using the constants for \textit{Ps. stutzeri} but realistic estimates of variations about these values were also considered.

Threshold number and inactivation constant were calculated using the batch inactivation data for \textit{Ps. stutzeri}. Fig. 7.2 shows how the threshold number and inactivation constant were derived from this data.
The threshold number, $n = 3$ is the intercept, on the y-axis, of the extrapolated straight line portion of the curve.

The inactivation rate constant, $k = 0.083 \text{ cm}^2 . \text{mW}^{-1} . \text{s}^{-1}$ is the slope of the straight line portion of the dose response curve.

### 7.2.2: Investigation of the Effects of Cellular Repair Mechanisms on Inactivation

All nucleic acid repair processes act in effect to counter disinfection treatments targeted against DNA. A useful way of accounting for repair processes was put forward by Novick and Szilard (1949), and required the estimation of so-called ‘Dose Modification Factors’ (DMF). The DMF may be defined as the ratio of the UV dose required to inactivate a certain fraction of a population of micro-organisms divided by the UV dose necessary to achieve the same effect when repair processes are in operation. A DMF of 1.0 would indicate that repair processes were inactive whereas DMF values less than 1.0 would be obtained if repair was taking place.
It is well known that cell counts for UV-irradiated liquids can be increased following irradiation by repair mechanisms (Mechsner et al., 1991; Liltved and Landfald, 1996; Baron and Bourbigot, 1996; Kashimada et al., 1996). However, samples of MWF which had undergone irradiation and which were subsequently held both in the light and the dark (as described in Chapter 3) failed to provide evidence that post-irradiation reactivation was occurring to any significant extent (see Table 5.4). This can not be taken as proof that the cells did not possess this ability, since it may have been subject to inhibition by one or more environmental factors. The latter may have been either physical (e.g. pH) or chemical, e.g. the presence in the MWF of a specific inhibitor of DNA repair. Such inhibitors have been reported in the past and include substances as diverse as caffeine and acriflavine (Harm, 1980). Under these circumstances therefore the DMF was taken to be 1.0. The issue of the repair capacity of pseudomonads is considered further in the Discussion.

7.2.3: Regrowth of the Treated Oil Emulsion

Prior to the irradiation of large batches of MWF, the microbial population had been allowed to adapt, i.e. grow in the medium. The rate of growth would have depended on a number of environmental factors, both physical ones, such as temperature and pH, but also those reflecting the composition of the environment.

In recirculatory treatment, such as was applied to the batches of MWF, cells would undergo irradiation and then be returned to the feed tank (see Fig. 3.8). Those cells not inactivated by exposure to UV would remain in the feed tank for a period of time determined by the recirculation rate and also the mixing regime inside the tank. During the time that cells remained in the tank they would begin to grow provided that conditions remained favourable.
In order to determine the rate of growth of cells in MWF, a portion of the fluid from the 1000 L experiment was removed and incubated as described in 3.1.3. The cell density against time was presented in Figure 5.7.

Data from the exponential phase of the growth curve was linearised and the microbial growth rate, $\mu = 0.093 \ \text{h}^{-1}$, was obtained from the slope of the straight line, giving a doubling time of approximately 7.5 hours.

7.2.4: Hydraulic Characterisation of the UV Contactor

Equation (2-9) is applicable only to reactors in which conditions approximate to those of complete mixing. Attempts were made to quantify the mixing regime in bells by conducting tracer studies (see Appendix F.1) but the data obtained was rendered unusable because of the relatively large hold-up time of liquid in the contactor following bell formation. Notwithstanding, it was assumed that perfect mixing prevailed to enable predictions to be made.

The residence time, $t$, was determined using the Lance and Perry model described in Chapter 6. For a flowrate of 1200 L/hr. and a nozzle gap opening of 3.0 mm, the residence time was 0.28 sec.

7.2.5: Intensity Determination

Intensities were predicted using the intensity model described in Chapter 6 for the special case of multi-lamp irradiation. The decadic absorption coefficient of the MWF at 253.7 nm was $\alpha = 109 \ \text{cm}^{-1}$ which is considerably higher than that of mains water (0.18; Shama, 1992a). Average intensities ranging from 0.22-0.29 mW/cm$^2$ were obtained.
7.2.6: Uniting Single Pass Inactivation with Regrowth

A computer program (inact.bas, see Appendix F.2) was written to combine equation (2-9) with other factors affecting the microbial population level of a continuously-irradiated liquid. Simply, if \( N_s \) is the surviving fraction after \( x \) passes through the contactor, the cell population before the \((x+1)^{th}\) pass will be equal to:

\[
(N_s \times \text{DMF}) + ((N_s \times \text{DMF}) \times \mu \times \text{CT})
\]  

(7-1)

where DMF = dose modification factor, \( \mu = \) specific growth rate, and CT = time taken to irradiate the entire batch (total volume/volumetric flow rate).

CT ranged from 0.17 h for the 200 L experiment to 0.83 h for the 1000 L experiment.

7.3: RESULTS

When values of intensities predicted by the model were substituted into equation (2-9), the reduction in cell viability obtained was very low, even for the 200 L experiment. The data suggested that the population returned to its original value in the interval between passes. In effect this would mean that the treatment had no effect on the microbial population of the emulsion, a prediction not borne out by experimental findings.

However, by rearranging (2-9), it was possible to estimate the average intensity from experimentally-obtained single-pass fractional survival figures obtained using MWF (see Table 5.4). Estimates obtained in this way gave average intensities of 61.7, 69.3 and 33.2 mW/cm² for 200, 400 and 1000 L.
experiments respectively. These values were then used to estimate viability reduction and the results are shown in Figs. 7.3-7.5.

**Fig. 7.3: Experimental Values From 200 L Continuous Irradiation Experiment Compared with Modelled Values**

![Graph showing experimental and modelled values over time](image)
Fig. 7.4: Experimental Values From 400 L Continuous Irradiation Experiment Compared with Modelled Values

![Graph showing experimental values compared with modelled values for 400 L experiment.]

Fig. 7.5: Experimental Values From 1000 L Continuous Irradiation Experiment Compared with Modelled Values

![Graph showing experimental values compared with modelled values for 1000 L experiment.]

Fig. 7.3 shows that the inactivation model closely predicts the inactivation of the 200 L batch of MWF. The results for the 400 L experiment (see Fig. 7.4)
show even closer agreement. In Fig. 7.5, however, predicted inactivation deviates significantly from the experimental data.

7.4: DISCUSSION

7.4.1: Intensity Predictions. Model Predictions and Parameter Testing

Significant discrepancies between predicted UV intensities and those deduced from experimental measurements based on single pass viable count reductions were apparent. Similar comparisons with purely aqueous systems described in Chapter 6 revealed, in contrast, generally good agreement. This is strongly suggestive that the discrepancies were due to the nature of the MWF itself. The MWF used here for the large scale disinfection studies (Hocut B200) was an oil in water emulsion with a relatively high (≈ 109 cm⁻¹) absorption coefficient as measured using a spectrophotometer. This coefficient was used directly in Lambert’s Law as part of the computations undertaken to estimate the UV intensity in the liquid film. However, direct measurement using a spectrophotometer will not distinguish between light failing to reach the detector because of absorption by the sample and that failing to do so due to scattering effects (the milky appearance of emulsions is in fact due to the phenomenon of light scattering). This is an important distinction, as if a fraction of the spectrophotometrically measured extinction were due to scattering rather than absorption, the scattered UV light would still retain the ability to exert a lethal effect towards organisms in suspension in the emulsion; Jagger (1967) stated that light scattering does not normally involve molecular absorption.

Some evidence for this has been obtained by Shama (unpublished) who found that aqueous suspensions of talc at concentrations of up to 4.25 g/L actually increase the disinfection efficiency compared to pure water. It has been suggested that the talc particles (approx. 9-12 μm in diameter) scatter light
within the thin liquid film and that as a consequence, the probability of an organism being struck by a UV photon is increased. Although the size of the oil droplets comprising the emulsions was approximately 2-3 μm, smaller than the talc particles, considerable scatter could still have occurred. Indeed, Stamatakis et al. (1990) conducted studies which showed that smaller particles were more efficient than larger particles at scattering UV. Jagger (1967) did propose what he claimed was a simple spectrophotometrically-based method for distinguishing between scattering and absorption, but the method yielded highly inconsistent results when applied to MWF emulsions. It is clear from the foregoing that additional work needs to be undertaken in order to obtain quantitative estimates of light scattering and absorption by oil in water emulsions.

Better agreement with experimental data was obtained by using single pass survival figures to estimate UV intensity, however intensity estimates for the 1000 L experiment (33.2 mW/cm²) seem anomalously low by comparison with values for the 200 and 400 L batch experiments (61.7 and 69.3 mW/cm² respectively). Whilst the microbial populations comprising the three batches were not identical, and some variation in single pass survival between batches was to be expected, the low value obtained for the 1000 L experiment would imply a significantly different microbial composition, an implication not borne out by the experimental findings (see Tables 5.1-5.3). Under these circumstances, therefore, it appeared justifiable to attempt to remodel disinfection of the 1000 L batch using the nominal intensity figure of 60 mW/cm². Fig. 7.6 shows the inactivation curve generated, along with that for the lower intensity value. It is immediately apparent that this recalculation results in a fit to experimental data similar to that for the 200 and 400 L experiments (see Figs. 7.3 and 7.4).

Using the calculated values of k, n and μ, an average intensity figure of 60 mW/cm², and taking N₀ to be equal to 10⁷ CFU/ml, the computer program
predicted that the pilot-scale apparatus is capable of disinfecting up to 3200 L of contaminated 4% Hocut B200 emulsion under the same conditions as those under which the continuous irradiation experiments were conducted (i.e. flow rate of 1200 L/hr., nozzle gap 3 mm).

Fig. 7.6: Comparison of Model Prediction for Average Intensities of 33.2 and 60 mW/cm² with Experimental Values

![Graph showing comparison between model predictions and experimental values.]

It was stated earlier in the Introduction that this attempt to model the disinfection process was preliminary, and that by implication, further experimental data would be required to enable predictions to be made with greater confidence. This is largely because the microbial population in the contaminated MWF were diverse and likely to change during the course of irradiation treatment. It seemed appropriate, therefore, to undertake a sensitivity analysis in which the effects of varying the parameters in equation (2-9) was considered. The values used in this analysis were based on experimental data from the literature. In the case of the specific growth rate, \( \mu \), highly relevant data on the growth of pseudomonads on fuel oil was found (Litchfield, 1979). However, kinetic inactivation data was considerably rarer
and ultimately data could only be found for a rather diverse collection of organisms. Notwithstanding, these represent a range of values of inactivation constants actually displayed by living organisms and in the absence of more specific data, they were used in the analyses presented below.

Figs. 7.7-7.9 show comparisons of experimental data points with model predictions made using different values of $\mu$, $k$ and $n$ for the 1000 L experiment (using 60 mW/cm$^2$ as the average intensity in the bell). In each case, the experimental data and model predictions shown cover only the first 50 hours of the experiment in order to clarify as much as possible the resulting figures. For $\mu$, the values used are those directly measured for the oil emulsion (see Fig. 5.7) and also the upper and lower values of the range published for the growth of pseudomonads in fuel oil (mentioned above). For $k$, the values used comprise that determined experimentally for $Ps. stutzeri$ and the published values for $E. coli$ and the f2 virus (see Table 7.1). For $n$, the values used are that for $Ps. stutzeri$ (3) and two arbitrarily chosen values (1 and 4), suitable published data not being available.
Fig. 7.7: The Effect of Variation of Specific Growth Rate on Model Predictions

![Graph showing the effect of variation of specific growth rate on model predictions.](image)

Fig. 7.7 shows that the measured value of $\mu$ for the oil emulsion system, 0.093, produces a good fit to the experimental data. The lower of the published figures, 0.12, produces a similar or slightly better fit, whilst 0.25 (the upper published figure) causes the model to predict a much shallower inactivation curve. It is important to note that $\mu$ will increase with temperature within the range in which an organism or population can grow. The implication of this is that, although UV inactivation is itself rather insensitive to temperature variations (Severin et al., 1983b; Abu-ghararah, 1994), variable regrowth rates mean that temperature can be important in UV disinfection by recycle.

Table 7.1: Measured Values of $k$ for Various Organisms

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>$k$, cm$^2$.mW$^{-1}$.s$^{-1}$</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed culture (oil emulsion)</td>
<td>0.0830</td>
<td>This work</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.8930</td>
<td>Severin et al., 1983a;</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>0.4330</td>
<td>Severin et al., 1984a.</td>
</tr>
<tr>
<td><em>f2 virus</em></td>
<td>0.0724</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 7.8: The Effect of Variation of $k$ on Model Predictions

![Graph showing the effect of variation of $k$ on model predictions.](image)

Fig. 7.9: The Effect of Variation of Threshold Number on Model Predictions

![Graph showing the effect of variation of threshold number on model predictions.](image)

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Fig. 7.8 shows that the inactivation rate constant value used (that of *Ps. stutzeri* as a typical pseudomonad) can be used in the model to predict an inactivation curve which fits experimental data well. The k value for the f2 virus, being similar to that of *Ps. stutzeri*, produced a very similar curve. When the constant for *E. coli* (an order of magnitude greater than for the other two organisms) was used, a rate of inactivation much greater than was actually observed was predicted. The steeper the slope of the linear portion of an organism’s dose response curve, the greater the value of k, and the faster the rate of inactivation (all other factors being equal).

Fig. 7.9 demonstrates the effect of variation of the threshold number (‘n’). The threshold number represents the number of photon hits theoretically required on the ‘target area’ (i.e. the DNA) of an organism in order that it be inactivated. This number is more generally taken to represent the ability of an organism to repair UV damage. Since n is present as exponent in equation (2-9), it is not surprising that small variations of this quantity produce widely differing predictions for the inactivation curve. The measured value for *Ps. stutzeri* (3) produced the best fit to the experimental data.

A value of 1 for n (used as the lowest value in Fig. 7.9) would be unlikely to apply to the mixed culture in the emulsion in reality, assuming that at least some of the organisms present have repair characteristics similar to *Ps. stutzeri*. This is because a value of 1 for n represents the case where there is no initial shoulder on an organism’s dose response curve (and, by implication, the organism possesses no repair capability), often the case for viruses. The distinction should be made between microbial reactivation following post-irradiation liquid holding, and repair ability as indicated by a shouldered dose response curve. The batch dose response curve of *Ps. stutzeri* (see Fig. 4.1) was obtained by plating samples of UV-treated cell suspension immediately after irradiation. The shoulder seen in Fig. 4.1 represents repair
of UV damage that occurs effectively ‘instantaneously’. Under laboratory conditions, *Ps. stutzeri* has been shown capable of further cellular reactivation following post-irradiation liquid holding. The implications of the failure of the mixed culture in the oil emulsion to exhibit liquid holding repair are discussed below.

Values of \( n \) of 5 or more resulted in predicted single pass inactivation figures so small that the population was predicted to have recovered to its original level before the whole volume of treated liquid passed through the contactor again (i.e. there would be no overall inactivation).

The finding that the microbial population was apparently unable to conduct post-irradiation repair in the environment of the MWF was unexpected. As previously mentioned, *Ps. stutzeri* is able to perform post-irradiation repair of UV-induced damage both in the dark and under photoreactivation conditions (illumination by near UV light). The organism was irradiated as a suspension in physiological saline solution, and the cell counts immediately after irradiation compared with those which were held in the dark and which were exposed to photoreactivating light (see 3.3). For dark repair a constant DMF of 0.8 was obtained, whilst for light-induced repair the DMF ranged from 0.41 to 0.53 (Peppiatt and Shama, unpublished).

In further experiments, batches of cell suspensions of *Ps. stutzeri* of 20 and 40 L were irradiated by recycle using the laboratory-scale apparatus. It was found that intermittent exposure to UV of the bacteria resulting from this form of treatment, enabled the cells to conduct extensive repair in the intervals between exposure (the cells were prevented from growing as they were maintained in suspension in physiological saline). This repair was also characterised in terms of DMF. The latter were shown to be volume-dependent and values as low as 0.39 were obtained. These results strongly indicate that the pseudomonads, the most prominent group present in the
contaminated MWF, possess mechanisms for post-irradiation repair, but that these were inhibited by environmental factors. In related work, Chan and Killick (1995) observed that repair processes in *E. coli* could be severely inhibited by concentrated saline. It would indeed be interesting to see whether a dose response curve obtained for *Ps. stutzeri* grown in MWF exhibited a shoulder, or if the MWF environment inhibits cellular DNA repair mechanisms completely.

7.4.2: Hydraulic Parameters

Salt tracer methods have been favoured in evaluations of the mixing characteristics of UV contactors (Thampi and Sorber, 1987; Thampi, 1990). The various flow parameters computed in the salt tracer experiments conducted here were those described by Scheible (1987), see Appendix F.1.

The theoretical retention time, $T$, for the water bells generated here was variable, but for most of the configurations used in this work was approximately equal to 0.25 seconds. Clearly the average values for $t_p$ (the time taken for the peak salt concentration to be reached) of 22.6 seconds (see Appendix F.1) for the 600 L/hr bell and 19.7 seconds for the 700 L/hr. bell do not reflect the time taken for any particular element to pass from the top to the bottom of the bell, but rather the time taken for its passage from the injection point just upstream of the entrance to the nozzle to the drainage point in the base of the contactor chamber (see Fig. 3.10). The hydraulic parameters quoted are therefore more a measure of the time taken for treated liquid to clear the contactor than of the actual residence time in the bell. Since, due to the very nature of a water bell, a continuous column of liquid does not exist at the instant the bell reaches the bottom of its fall, it would be very difficult to accurately measure a tracer peak at that point.
Severin and co-workers (Severin 1982; Severin et al., 1984a; Severin et al., 1984b) found good experimental agreement for their model predictions which indicated that plug flow conditions produce the best inactivation efficiency in flow-through UV contactors. They concluded that mixing in the radial direction is beneficial for UV inactivation, whilst mixing in the longitudinal direction is detrimental to inactivation efficiency.

The mixing regime in the feed tank was mentioned earlier as exerting an influence on the residence time of individual cells in between episodes of exposure to UV. No attempt was made to characterise the regimes during irradiation of the MWF batches and in further developments of the model this issue would need to be addressed.

7.5: CONCLUSIONS

Indiscriminate use of the limited experimental data available gave model predictions which were clearly not representative of the experimental evidence which had been accumulated. Better predictions were achieved by using single pass survival measurements to predict dose by means of a rearranged form of equation (2-9). However, it is felt that the modelling exercise highlighted areas in which additional experimental data needs to be obtained and as such, was a valuable exercise.

In any recirculating form of UV treatment control or reduction of the microbial population will only be achieved if the rate of inactivation exceeds that of growth/repair. The pilot-scale apparatus is capable of disinfecting volumes of up to 3200 L under the same conditions as those for the contaminated oil emulsion system.
Although important in many industrial situations and in the types of organism encountered in the MWF system, post-irradiation repair of UV-induced damage did not appear to occur.
CHAPTER 8: CONCLUSIONS AND SUGGESTIONS
FOR FURTHER WORK

8.1: UV DOSE MEASUREMENT

UV dose estimates were obtained for pilot and laboratory scales of UV contactor using potassium ferrioxalate actinometry and bioassays based on *Ps. stutzeri* and a repair-deficient strain of *E. coli*. There was good agreement between the estimates produced using the *E. coli* 11190 bioassay and the potassium ferrioxalate actinometer. This was attributed to the fact that *E. coli* displayed linear dose-response characteristics. Further evidence for the validity of dose estimates based on these two methods was provided by calculating doses using intensity estimates obtained with a UV radiometer. The estimates from the *Ps. stutzeri* bioassay were much higher than those produced by all the other methods evaluated. This was because the doses measured did not coincide with the linear portion of the organisms' dose response curve.

Estimates for the dose applied by the pilot-scale apparatus did not rise linearly when the number of UV sources used was increased. One possible explanation for this was that there was interference between one lamp and another. No previously published evidence confirming the existence of this phenomenon has been found in the literature, however there is now a case for further examining these putative interference effects. This could be done by irradiating a bell using external sources only and comparing the dose estimates when the lamps were separated from each other by baffles and those made when there were no baffles (as was the case in this work). It would also be advisable to attempt the evaluation of the amount of light reflected back from the surface of bells. This could be achieved by placing sensitive photometric sensors externally to the bells in locations such that they would only receive reflected UV light.
Whilst good agreement was obtained with identical batches of potassium ferrioxalate solutions, variations occurred between batches. It was suggested that this might have been caused either by the presence of chemical species affecting the conversion of ferric ions or alternatively, that the observed variations were due to the effects of ambient temperature on the spectral emission of the UV lamps. Potassium ferrioxalate has proved to be a popular actinometer in recent times, but it might prove worthwhile evaluating an alternative actinometer such as uranyl oxalate to establish whether better consistency could be achieved.

Despite the measures which were taken to ensure that test suspensions of *Ps. stutzeri* and *E. coli* were generated and handled with consistency, variations between batches of cells were nevertheless apparent. These difficulties could possibly be overcome by using spores which might be expected to display less variability than vegetative cells. In order to enable dose estimates to be made with precision, an absolute requirement would be that the spores display linear dose response characteristics for the dose range to be tested.

8.2: DISINFECTION OF METAL WORKING FLUIDS

The bell contactor was effective in reducing the microbial loading of badly infected Metal Working Fluid (MWF) oil emulsions to stable low levels. Indeed, the treatment reduced microbial counts to near sterility in the 400 and 1000 L experiments. The levels of microbial contamination (10⁴-10⁷ CFU/ml) prior to each experiment were equivalent to those seen in industry for a typical case of serious infection (Mattsby-Baltzer et al., 1989). Such a reduction should diminish significantly the infection risk to machine operators, and may extend the life of the emulsion itself. It is felt highly desirable that work should be done (in parallel with untreated controls) on the continuous
irradiation of freshly prepared (i.e. essentially free of microbial contamination) batches of metalworking fluid. Such a strategy may demonstrate the ability of the system to keep the MWF free from developing persistent microbial contamination.

Evidence was obtained to show that fouling of the quartz thimbles shrouding the UV lamps occurred. The fouling was more pronounced on the external lamps. Fouling is not seen as an insurmountable problem and some relatively straightforward design modifications could be put into place to reduce the incidence of fouling. One such method would be to simply change the position of the lamps in relation to the bell e.g. raising them to avoid liquid droplets becoming deposited on them. These droplets, it will be remembered, were produced by the impact of the bell on the free liquid surface. Alternatively, attention could be devoted towards designing a shield which would not itself interfere with the irradiation of the bell but would prevent droplet deposition on the sources.

Isolation and identification of the micro-organisms present in the MWF showed the majority (both in species and numbers) of them to be pseudomonads. This is in agreement with the findings of other authors (King and McKenzie, 1977; Mattsby-Baltzer et al., 1989; Rossmore, 1993). *Ps. aeruginosa* was the commonest organism for both the 400 and 1000 L experiments.

Attempts were made to monitor the fates of individual bacterial species present in the MWF during UV treatment. Some success was achieved in so far as it was revealed that certain species, notably *Ps. aeruginosa*, seemed to persist throughout the entire period of treatment, whereas others e.g. *Sphingomonas paucimobilis*, were only detected over relatively short periods. It was felt that the effort required to identify every colony noted during the
continuous irradiation experiments would far outweigh any benefit gained thereby.

The mixed microbial populations present in the MWF were unable to conduct either light or dark repair under the environmental conditions prevailing in the MWF. However, regrowth of elements of the population occurred once UV irradiation had ceased.

There was no detectable variation in the corrosion inhibition capability of the MWF investigated over the period of the disinfection experiments, but an interesting new method of quantitatively evaluating corrosion was developed.

8.3: UV DOSE ESTIMATION

The hydrodynamic model of Lance and Perry (1953) enabled the shape, liquid velocity and film thickness of the bells generated using the annular jet nozzle to be predicted. Estimates for these parameters obtained using this model were in close agreement with experimentally determined values. Experimental investigations (Gasser and Marty, 1994) show that, in situations where internal overpressure causes the Lance and Perry model to predict spiralled bell profiles, the profiles produced in nature are the same as predicted, but with the 'loops' excised. Consequently it would be useful to amend the computer program (belldose.bas) so that these loops were excised.

The UV intensity field in the vicinity of UV lamps was modelled using a volumetric emission model. This was combined with the hydrodynamic bell model to yield estimates of UV doses delivered to bells. The model produced dose estimates which were generally in close agreement with those produced by both bioassay and actinometric methods.
In particular, the results obtained using the model were sufficiently encouraging to enable model estimates to be used with greater confidence. This would enable, for example, operating regimes resulting in maximum UV doses to be identified and subsequently verified, resulting in a reduced programme of experimentation.

8.4: DISINFECTION MODELLING

An initial attempt at modelling the disinfection kinetics of batch irradiated systems was presented in Chapter 7 with specific reference to MWFs. Whilst apparently yielding acceptable results under certain conditions, the exercise primarily served to highlight areas where further data would be required to enable predictions to be made with greater confidence. Areas requiring particular attention are considered below.

The expression used to model the re-growth of the microbial population during the time interval between passes through the contactor (Equation 7-1) assumed a growth rate of $\mu CT$ in conditions where $N_x$ (the number of surviving organisms after $x$ passes through the contactor) remained approximately constant during the interval $CT$. In situations where $CT$ is very large, however, this assumption would become invalid: i.e. the population size may increase significantly in the interval between irradiation events.

Efforts were made to establish whether conditions inside the liquid films approximated those of perfect mixing in order to use a series-event kinetics rate model (Equation 2-9) for modelling cell inactivation. The conductivity technique chosen (see Appendix F and Chapter 7) proved inappropriate and it was impossible to characterise the mixing regime. Notwithstanding, series-event kinetics were applied in the absence of firm evidence that mixing inside the films was not complete. This strategy had the advantage of allowing some progress to be made with the model and of enabling a sensitivity
analysis to be conducted. It is felt that, if the model were to be further developed, the characterisation of the mixing regime would need to be determined. In this regard, laser doppler velocimetry might prove the best method for the direct determination of the flow patterns within the liquid films constituting the bells.

The mixing regime within the system as a whole (i.e. of the bulk liquid in the main tank, and that held-up in other parts of the apparatus) was not assessed. In the preliminary model described in Chapter 7, it was necessary to assume complete mixing, and therefore that all members of the microbial population pass the UV sources after the time interval CT. The volume of liquid treated was increased from 200 to 400 and finally 1000 L, and it is unlikely that (in the main tank at least) the mixing characteristics were the same in all cases, however. In any further studies of this kind it might prove useful to attempt to establish similar mixing patterns at each scale of operation. This could be achieved by the addition of an impeller to the main tank.

Further studies are needed to identify methods which could allow a true measure of the absorption coefficients of oil emulsions to be made, accounting for the effects of light scattering. Such data would significantly improve the accuracy of predictions obtainable with the simplified disinfection model.

Whilst clearly impossible to obtain inactivation characteristics on each species present in the diverse microbial populations present in MWFs, it is clear that more experimental data is required. It may prove the case that restricting such an exercise to a small handful of the most prominent members of the microbial population may yield improved predictions. This work could lead to the identification of a small group of 'indicator organisms', such as is used in water treatment, on which further studies could be concentrated.
Although often reported as a potential disadvantage of UV treatment, post-irradiation repair mechanisms were apparently not in operation during the irradiation of contaminated MWF.

8.5: MICROBIOLOGICAL SAFETY

A number of specific safety precautions were taken during experiments in which industrially supplied contaminated MWFs were employed (see Chapter 5) in addition to the practices routinely employed in handling microbially contaminated liquids. These were:

(i) The physical siting of the pilot plant UV contactor in a remote laboratory area.
(ii) The complete envelopment of the bell within a UV-opaque curtain, which in addition to guarding against any UV hazards, contained any aerosols which may have been formed.
(iii) The location of the experimental equipment described in Fig. 3.8 within a mild steel bund capable of holding 1½ times the largest volume (1000 L) used in the event of leakage from any part of the system.
(iv) The imposition of a strictly limited time intervals over which experiments with 'industrially' contaminated MWFs were conducted.
(v) The immediate transfer to closed containers of the entire batch of MWF on completion of experimental work for disposal by a company officially registered for the disposal of waste waters.

During the course of the experimental work referred to above, a number of spoilage microorganisms were isolated and identified. amongst these was *Ps. aeruginosa*, an organism that has routinely been reported by numerous other workers as a common contaminant of MWFs (Sondossi *et al.*, 1985a,b; Foxall-Van Aken *et al.*, 1986). It was not initially appreciated that this organism had been classified by the Advisory Committee on Dangerous
Pathogens as belonging to Hazard Group 2 at a time shortly before these experiments were actually being conducted. The formal definition of this category is given as ‘a biological agent that can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or effective treatment available’ (Anon., 1995). *Ps. aeruginosa* was not inoculated into the test emulsion, but appeared as an adventitious contaminant during the first experiment, and was subsequently isolated regularly during the remainder of that work. *Ps. aeruginosa* has been found as a spoilage organism in meat and milk, giving rise to so-called ‘blue milk’; in man it is generally found as a secondary infection (e.g. in hospitals), but it has been reported, in rare cases, as a cause of blindness and even death (Collins and Lyne, 1984).

In consideration of the above, it is strongly recommended that if any future experimental studies *Ps. aeruginosa*, or indeed any other organism belonging to Hazard Group 2, is isolated, then the MWF from which it originated should immediately be disposed of in an approved manner.
APPENDIX A

List of Symbols, Units and Abbreviations Used

\( b \) = Absorption coefficient.

\( C \) = Sterilant concentration.

\( \text{CFU} \) = Colony forming units.

\( \text{cm} \) = Centimetre(s).

\( \text{cm}^2 \) = Square centimetre(s).

\( \text{cm}^3 \) = Cubic centimetre(s).

\( \text{CT} \) = Time taken to irradiate whole batch of liquid.

\( ^\circ \text{C} \) = Degrees centigrade.

\( \text{D} \) = Dose.

\( E_x \) = Longitudinal dispersion.

\( g \) = gram(s).

\( g \) = Acceleration due to gravity.

\( h \) = Thickness of bell sheet.

\( h \) = Hour.
hr. = Hour(s).

I = Light intensity.

$I_0$ = Incident light intensity.

$\bar{I}$ = Average light intensity.

$i$ = Event level (series-event kinetics).

IBRG = International Biodeterioration Research Group.

$k$ = Inactivation constant.

$L$ = litre(s).

$L$ = Number of cells in a clump.

$l$ = Thickness of irradiated medium.

$M$ = Molar.

$M_i$ = An organism which has reached event level $i$ (series-event kinetics).

$m$ = Dimensionless quantity, ratio of average light intensity to light intensity at surface of quartz lamp sheath.

$m$ = Metre(s).

$m^3$ = Cubic metre(s).
min. = Minute(s).

ml = Millilitre(s).

mm = Millimetre(s).

mW = Milliwatt(s).

\(N_0\) = Number of viable cells at time zero.

N = Number of viable cells.

n = Threshold number.

NCIMB = National Collection of Industrial and Marine Bacteria.

nm = Nanometres.

Pa = Pascal(s).

PTFE = Polytetrafluoroethylene.

Q = Mass flow rate.

R = Radial co-ordinate in Lance and Perry model.

\(R_e\) = Reynolds number.

\(R_h\) = Hydraulic radius ratio.
rpm = Revolutions per minute.

s = Second(s).

T = Theoretical retention time.

TN = Surface tension.

t = Exposure time.

$\text{t}_p$ = Time taken for tracer peak to be reached.

$\text{t}_{10}$ = Time taken for 10% of tracer to pass.

$\text{t}_{90}$ = Time taken for 90% of tracer to pass.

$\text{t}_{90}/\text{t}_{10}$ = Morrill dispersion index.

v = Velocity.

$v_o$ = Initial velocity.

W = Watt(s).

Z = Longitudinal co-ordinate in Lance and Perry model.

$\zeta$ = Angle of light path to the vertical.

$\theta$ = Angular co-ordinate of a bell element.

$\lambda$ = View factor angle.
\( \mu = \) Specific growth rate.

\( \mu l = \) microlitre(s).

\( \mu W = \) Microwatt(s).

\( \pi = \) Pi.

\( \rho = \) Density.

\( \sigma_{o1} = \) Standard deviation (small population).

\( \tau = \) Theoretical retention time.

\( \Phi = \) Quantum yield.

\( \phi = \) Half of angle \((2\phi)\) moved along arc in solution to differential equation of bell surface.

\( \psi = \) Angle formed by tangent of bell surface with the horizontal.
APPENDIX B

Culture Media

1) Agar No. 1 (Oxoid)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>7.0 % w/w</td>
</tr>
<tr>
<td>Ash</td>
<td>2.0 % w/w</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>&lt;0.1 % w/w</td>
</tr>
<tr>
<td>NaCl</td>
<td>&lt;0.1 % w/w</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>&lt;0.005 % w/w</td>
</tr>
<tr>
<td>CO₃</td>
<td>0.9 % w/w</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>0.1 % w/w</td>
</tr>
<tr>
<td>Ca</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Mg</td>
<td>40 ppm</td>
</tr>
<tr>
<td>Fe</td>
<td>80 ppm</td>
</tr>
</tbody>
</table>

2) Egg Yolk Agar (Harrigan and McCance, 1976)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient agar (Oxoid)</td>
<td>100 ml.</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0 g.</td>
</tr>
<tr>
<td>Egg yolk emulsion</td>
<td>10 ml.</td>
</tr>
</tbody>
</table>

(a) The salt was added to the dissolved nutrient agar, which was boiled until all the agar was melted, and then autoclaved for 20 minutes at 121 °C.
(b) The autoclaved agar was cooled to 45 °C in a water bath, at which point 10 ml of sterile egg yolk emulsion was aseptically added to the agar. After mixing, the agar was poured aseptically into sterile petri dishes, which were allowed to cool and solidify before being dried for 45 minutes in a laminar flow cabinet.

3) Egg Yolk Emulsion (Harrigan and McCance, 1976)

(a) The yolks were removed from the whites of eggs using a pipette. Four parts distilled water were added to one part of egg yolk, and the whole was mixed together.
(b) The mixture was heated in a water bath at 45 °C for 2 hours.
(c) The emulsion was centrifuged for 15 minutes (5000 rpm, 25 °C) to remove the precipitate.

(d) The supernatant was first filtered through a 1 μm membrane filter (Whatman), and finally sterilized by passage through a 0.2 μm sterile filter (Whatman) into a sterile vial.

4) 'Lab-Lemco' Powder (Oxoid)

<table>
<thead>
<tr>
<th>Component</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
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<td>Ash</td>
<td>15.6</td>
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<tr>
<td>NaCl</td>
<td>7.2</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.7</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>12.0</td>
</tr>
<tr>
<td>Amino nitrogen</td>
<td>1.1</td>
</tr>
<tr>
<td>Ca</td>
<td>300</td>
</tr>
<tr>
<td>Mg</td>
<td>200</td>
</tr>
<tr>
<td>Cu</td>
<td>1ppm</td>
</tr>
<tr>
<td>Fe</td>
<td>112</td>
</tr>
</tbody>
</table>

pH 7.2 ± 0.2

5) MacConkey Agar No. 3 (Oxoid)

<table>
<thead>
<tr>
<th>Component</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>20</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
</tr>
<tr>
<td>Bile Salts No. 3 (Oxoid)</td>
<td>1.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>5</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.03</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
</tr>
</tbody>
</table>

pH 7.1 ± 0.2

Sterilize by autoclaving at 121 °C for 15 minutes.
6) **Nutrient Agar**

**FORMULA**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Lab-Lemco' Powder</td>
<td>1</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>2</td>
</tr>
<tr>
<td>Peptone</td>
<td>5</td>
</tr>
<tr>
<td>NaCl</td>
<td>5</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
</tr>
</tbody>
</table>

pH 7.4 ± 0.2

Sterilize by autoclaving at 121 °C for 15 minutes.

7) **Nutrient Broth (Oxoid)**

**FORMULA**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Lab-Lemco' Powder</td>
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</tr>
<tr>
<td>Yeast Extract</td>
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</tr>
<tr>
<td>Peptone</td>
<td>5</td>
</tr>
<tr>
<td>NaCl</td>
<td>5</td>
</tr>
</tbody>
</table>

pH 7.4 ± 0.2

Sterilize by autoclaving at 121 °C for 15 minutes.

8) **Phosphate Buffered Saline (Dulbecco 'A': Oxoid)**

**FORMULA**

<table>
<thead>
<tr>
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<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.15</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.2</td>
</tr>
</tbody>
</table>

pH 7.3

Sterilize by autoclaving at 115 °C for 10 minutes.

9) **⅓ Strength Ringer Solution (Ringer Solution):**

**FORMULA**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>2.25</td>
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<tr>
<td>KCl</td>
<td>0.105</td>
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<tr>
<td>CaCl₂·6H₂O</td>
<td>0.12</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.05</td>
</tr>
</tbody>
</table>

pH 7.0

Sterilize by autoclaving at 121 °C for 15 minutes.
10) **Sabouraud Dextrose Agar (Oxoid)**

**FORMULA**

- Mycological Peptone (Oxoid) \( \text{10 g/L} \)
- Dextrose \( \text{40 g/L} \)
- Agar No. 1 \( \text{15 g/L} \)

\[ \text{pH 5.6} \pm 0.2 \]

Sterilize by autoclaving at 121 °C for 15 minutes.

11) **Tryptone Soya Agar (TSA):**

**FORMULA**

- Tryptone \( \text{15 g/L} \)
- Soya Peptone \( \text{5 g/L} \)
- NaCl \( \text{5 g/L} \)
- Agar \( \text{15 g/L} \)

\[ \text{pH 7.3} \pm 0.2 \]

Sterilize by autoclaving at 121 °C for 15 minutes.
### APPENDIX C.1

Batch Dose Survival Data for Bioassay Curves

#### Table C.1.1: Data For The *Ps. stutzeri* Bioassay Curve

<table>
<thead>
<tr>
<th>Exp. Group</th>
<th>Dose (mW-s/cm²)</th>
<th>Fractional Survival (N/N₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>0.902</td>
<td>0.880</td>
</tr>
<tr>
<td></td>
<td>1.805</td>
<td>0.660</td>
</tr>
<tr>
<td></td>
<td>2.707</td>
<td>0.420</td>
</tr>
<tr>
<td></td>
<td>4.512</td>
<td>0.090</td>
</tr>
<tr>
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<td>0.090</td>
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<td></td>
<td>9.346</td>
<td>1.26E-5</td>
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<tr>
<td></td>
<td>11.682</td>
<td>1.12E-5</td>
</tr>
</tbody>
</table>

#### Table C.1.2: Data For The *E. coli* 11190 Bioassay Curve

<table>
<thead>
<tr>
<th>Exp. Group</th>
<th>Dose (µW-s/cm²)</th>
<th>Fractional Survival (N/N₀)</th>
</tr>
</thead>
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<tr>
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<tr>
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<td>1.13E-4</td>
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<td>4.25E-2</td>
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<tr>
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<td>59.12</td>
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<td></td>
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<td>5.88E-3</td>
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<td>177.32</td>
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APPENDIX C.2

Operating Data for Bell Contactors: Bioassay and Actinometry

Table C.2.1: Operating Data for the Laboratory-Scale Apparatus at 650 L/hr.

<table>
<thead>
<tr>
<th>Nozzle gap</th>
<th>1.9 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell volume</td>
<td>43.49 cm³</td>
</tr>
<tr>
<td>Inside Surface Area</td>
<td>1688 cm²</td>
</tr>
<tr>
<td>Mean Irradiated Depth (vol./area)</td>
<td>0.026 cm</td>
</tr>
<tr>
<td>Residence time</td>
<td>0.22 s</td>
</tr>
</tbody>
</table>

Table C.2.2: Operating Data for the Laboratory-Scale Apparatus at 860 L/hr.

<table>
<thead>
<tr>
<th>Nozzle gap</th>
<th>4.6 mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell volume</td>
<td>49.43 cm³</td>
</tr>
<tr>
<td>Inside Surface Area</td>
<td>1536 cm²</td>
</tr>
<tr>
<td>Mean Irradiated Depth (vol./area)</td>
<td>0.032 cm</td>
</tr>
<tr>
<td>Residence time</td>
<td>0.22 s</td>
</tr>
</tbody>
</table>

Table C.2.3: Operating Data for the Pilot-Scale Apparatus at 990 L/hr.

<table>
<thead>
<tr>
<th>Nozzle gap</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Bell volume</td>
<td>68 cm³</td>
</tr>
<tr>
<td>Inside Surface Area</td>
<td>3511 cm²</td>
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<tr>
<td>Mean Irradiated Depth (vol./area)</td>
<td>0.019 cm</td>
</tr>
<tr>
<td>Residence time</td>
<td>0.26 s</td>
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</tbody>
</table>

Table C.2.4: Operating Data for the Pilot-Scale Apparatus at 1080 L/hr.

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>Bell volume</td>
<td>85 cm³</td>
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<tr>
<td>Inside Surface Area</td>
<td>1453 cm²</td>
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<tr>
<td>Mean Irradiated Depth (vol./area)</td>
<td>0.059 cm</td>
</tr>
<tr>
<td>Residence time</td>
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</table>
APPENDIX D

Continuous UV Treatment of Hocut B200

Table D.1: Data for the Continuous UV Treatment of 200L Hocut B200

<table>
<thead>
<tr>
<th>Time (hr.)</th>
<th>CFU/ml</th>
<th>N/N₀</th>
<th>pH</th>
<th>Temp. °C</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.00</td>
<td>7.2</td>
<td>25.0</td>
</tr>
<tr>
<td>1</td>
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<td>-</td>
</tr>
<tr>
<td>2</td>
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<td>0.21</td>
<td>-</td>
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</tr>
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<td>3</td>
<td>1.2E6</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
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<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>7.0E5</td>
<td>0.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>5.8E5</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
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<td>0.03</td>
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<td>-</td>
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<td>-</td>
</tr>
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<td>-</td>
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<td>6.5</td>
<td>-</td>
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</table>

Table D.2: Data for the Continuous UV Treatment of 400L Hocut B200

<table>
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<tr>
<th>Time (hr.)</th>
<th>CFU/ml</th>
<th>N/N₀</th>
<th>pH</th>
<th>Temp. °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0E7</td>
<td>1.00</td>
<td>6.8</td>
<td>25.0</td>
</tr>
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<td>4</td>
<td>3.0E5</td>
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<td>-</td>
<td>27.5</td>
</tr>
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<td>6</td>
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<td>8.1E-3</td>
<td>-</td>
<td>29.5</td>
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<td>24</td>
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</tr>
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<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33.0</td>
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<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<td>31.0</td>
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### Table D.3: Data for the Continuous UV Treatment of 1000L Hocut B200

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<th>Time (hrs.)</th>
<th>CFU/ml</th>
<th>N/N₀</th>
<th>pH</th>
<th>Temp. °C</th>
</tr>
</thead>
<tbody>
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<td>-</td>
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<td>247</td>
<td>77</td>
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<td>-</td>
<td>34.0</td>
</tr>
</tbody>
</table>
APPENDIX E.1

Procedure for Modelling Bell Shape

The method is based entirely on the work of G.N. Lance and R.L. Perry (1953). Fig. E.1.1 shows the co-ordinate system used for this model.

**Fig. E.1.1. Tangential-Normal Co-ordinate System for Liquid Bell Model**

The quantity of liquid $Q$ flowing in any unit of time across a section AB perpendicular to the curve of the liquid bell will be constant, thus giving the continuity equation:

$$Q = 2\pi r h v p$$  \hspace{1cm} (E.1-1)

(Where $Q =$ mass liquid flow rate, $r =$ radius of bell nozzle, $h =$ thickness of bell sheet, $v =$ velocity of liquid at AB, and $p =$ density of the liquid). When the element of volume shown in Fig. E.1.1 is considered it will be seen that, since there is no resultant surface tension force in the tangential direction, the tangential equation of motion is:

$$h \delta s. \delta \theta p g \sin \Psi = h r \delta s. \delta \theta p v \, dv/ds$$

or,

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\[ g \sin \Psi = v \frac{dv}{ds} \quad (E.1-2) \]

(where \( g \) = acceleration due to gravity). The equation of motion for the normal direction can be written:

\[ \left( \frac{2T}{R} + \frac{2T \sin \Psi}{r} + \Pi - p \right) + \rho h g \cos \Psi = \frac{v^2}{R} \rho h \quad (E.1-3) \]

(where \( T \) = surface tension, and \( R \) = the radius of curvature at AB in the vertical plane). Equation (E.1-2) may be written as \( gdz = vdv \), and so by integration:

\[ 2gz = v^2 - v_o^2 \quad (E.1-4) \]

(where \( v_o \) = the initial velocity of the bell).

By writing \( \alpha = 2\pi (\Pi - p)/Qv_o \) and \( \beta = 4\pi T/Qv_o \), and using equation (E.1-1), equation (E.1-3) can be written as:

\[ v_o \beta \left( r \frac{d\Psi}{ds} + \sin \Psi \right) + \alpha rv_o + \frac{g \cos \Psi}{v} = v \frac{d\Psi}{ds} \quad (E.1-5) \]

If \( g \) is eliminated from equations (E.1-4) and (E.1-5), the equation which has been used for the value of surface tension can be obtained:

\[ T = \frac{Q}{4\pi} \left( \frac{-\Delta(v\cos \Psi)}{\Delta s - \Delta(r\cos \Psi)} \right) \quad (E.1-6) \]

(where \( \Delta = d/d\Psi \)).
To derive the differential equation of the bell surface, \( v \) must be eliminated from equations (E.1-4) and (E.1-5) to give:

\[
v_0 \beta \left( \frac{z''r}{(1 + z'^2)^{1.3}} + \frac{z'v_0}{(1 + z'^2)^{1.3}} \right) + \alpha rv_0 + \frac{g}{(1 + z'^2)^{1.3}(2gz + v_0^2)^{1.3}} = \frac{z''(2gz + v_0^2)^{1.3}}{(1 + z'^2)^{1.3}}
\]

If the substitutions \( \gamma = g/v_0^2 \) and \( e^2 = 1 + 2\gamma z \) are used, the equation above may be written as:

\[
1 + z'^2 \left\{ \left( \frac{\gamma}{e} \right) + \beta z' + \alpha r(1 + 2z'^2)^{1.3} \right\} = z''(e - \beta r)
\]

or,

\[
R \left( \beta \sin \Psi + \alpha r + \frac{\gamma \cos \Psi}{e} \right) = e - \beta r \quad \text{(E.1-7)}
\]

Equation (E.1-7) is a second order non-linear differential equation, and cannot be solved analytically. Several numerical solutions have been used, however.
Equation (E.1-7) gives an expression for the radius of curvature, $R$, directly. The numerical solution of an equation of the form

$$R = f(r, z, z')$$

(E.1-8)

is required. The method is based on Euler's polygon method for first order differential equations. In Fig. E.1.2 the co-ordinates of a point $P_o (r_o, z_o)$ are assumed known, as is the slope $\Psi_o$ of the curve at $P_o$. From (E.1-8) the radius of curvature at $P_o$ is calculated as $R_o = f(r_o, z_o, \tan(\Psi_o))$. If one imagines travelling around the arc of a circle, centre $C_o$ and radius $R_o$, through an angle $2\phi$, a point is arrived at whose co-ordinates are $r_o + 2R_o \sin\phi \cos(\Psi_o + \phi)$ and $z_o + 2R_o \sin\phi \sin(\Psi_o + \phi)$; the slope at this point is $\Psi_o + 2\phi$. Provided $\phi$ is small, one arrives at the point $P_1$ which is another point on the curve satisfying the differential equation (E.1-8). At each point, therefore, the radius of curvature, $R$, is calculated using equation (E.1-7), and used to find the co-ordinates of the next point using the general equation set:
\[
\begin{align*}
    r_{n+1} &= r_n + 2R_n \sin \phi \cos (\psi_n + \phi) \\
    z_{n+1} &= z_n + 2R_n \sin \phi \sin (\psi_n + \phi) \\
    \psi_{n+1} &= \psi_n + 2\phi 
\end{align*}
\] (E.1-9)

The arc length was set to a constant value, with \( \phi \) varying with changing radius of curvature. For each point the sheet thickness, \( h \), and the velocity were also calculated, using (E.1-1) and (E.1-4) respectively.
APPENDIX E.2

Calculation of View Factors

As stated in 6.4.1.1, the intensity of light incident at a surface may be generally expressed as:

\[ I = I_o \cos \lambda \]  

(E.2-1)

where \( I_o \) = the point intensity at a given point relative to a notional point source emitter in a lamp, and \( \lambda \) = the angle at which the light ray strikes the surface.

The quantity \( \cos \lambda \) is called the ‘view factor’. The view factor changes for each point source/bell element pairing. The calculation of the view factor is facilitated by the geometric argument shown in Fig. E.2.1.

Fig. E.2.1: Calculation of the View Factor Angle

It can be seen from Fig. E.5 that \( \lambda \) may be simply expressed as:

\[ \lambda = \zeta - \psi \]  

(E.2-2)
(Note that $\psi$ is generated by the Lance and Perry numerical method)

For every point source/bell element pairing $\zeta$ is calculated, enabling the calculation of $\lambda$ and the view factor. UV point intensity values calculated from (6-2) can thus be modified using (E.2-2) to give the average UV intensity at the surface of each bell element. Equation (E.2-2) is valid for all conditions encountered (i.e. point source $z$ co-ordinate greater or less than that of bell element centre, and for all values of $\psi$).

**Correcting for UV Absorption Within the Bell Element**

The absorption of UV light by the liquid of a bell element can be simply calculated by means of the expression known as Lambert’s Law:

$$ I(\rho) = I_0 \cdot e^{-\mu \rho} \quad (E.2-3) $$

where $I_0$ = incident UV intensity, $\rho$ = path length through liquid film, and $\mu$ = absorption coefficient.

The program calculates the UV intensity at evenly spaced points along the light path through the bell element using (E.2-3). The intensity reduction caused by absorption within an element also follows from (E.2-3):

$$ \left( \frac{I}{I_0} \right) = \frac{1}{N_T} \sum_{k=1}^{N_T} e^{-\mu \rho_k} \quad (E.2-4) $$

where $N_T$ = the number of points along the path length at which intensity is calculated.
APPENDIX E.3

The Determination of the Irradiated Sector of the Bell

Fig. E.3.1 shows the situation where an external light source irradiates a sector of the bell.

**Fig. E.3.1: Calculation of the Critical Angle**

By inspection of Fig. E.3.1, it can be seen that there is a critical angle beyond which light rays from the source will not impinge on the bell (i.e. there would be a view factor angle $\geq 90^\circ$. This critical angle, $\theta$, for any horizontal sector of the bell can be calculated as:

$$\theta = \tan^{-1}\left(\frac{\text{OPP}}{r}\right) \quad \text{(E.3-1)}$$

where $r = r$ co-ordinate of bell surface, and 0.1725 is the $r$ co-ordinate of the long axis of the external source in metres.

The critical angle corresponds to the central angle at which the output from the external source is tangential to the bell surface. Since the source will emit light either side of the centre line (see Fig. E.3.1), the critical angle is equal to
half the irradiated radial sector of the bell. If there are sixteen evenly-spaced external UV sources (as was the case with the pilot-scale apparatus), the bell will be evenly irradiated (i.e. no unirradiated portions) if \( \theta \) is \( \geq 11.25^\circ \) \((= 0.2 \text{ radians})\). In none of the cases modelled was the radius of the bell surface great enough at any point to produce values of \( \theta \leq 11.25^\circ \) \((\theta \leq 11.25^\circ \text{ when } r \geq 16.92 \text{ cm})\), so there was always irradiation overlap in this system. The symmetry in the system means that it is only necessary to determine the UV irradiation delivered by a single light source. Further, it is also theoretically acceptable to add the average intensity for external irradiation to that for the centrally-mounted lamp if the bell is irradiated from both sides simultaneously. This assumption means that the model fails to take into account any interference between different UV sources (as proposed in Chapter 4).
APPENDIX E.4

The Calculation of the View Factor Angle for Sources Outside of the Bell

In order to calculate the view factor angle in three dimensions, it is first necessary to determine the angles formed by both the vertical and horizontal components of the normal to the bell element surface with a line parallel to that which would join the long axes of the whole bell and the external lamp.

(a) **Vertical Direction**

**Fig. E.4.1: Calculation of Vertical Angle of Normal**

Fig. E.4.1 shows the calculation of the angle between the vertical component of the normal to the bell element surface and the horizontal. For case (a), where the surface of the bell is sloping away from the lamp, the angle is equal to $\psi/2 - \psi$. For case (b), where the surface of the bell is sloping towards the lamp, the angle is equal to $\psi - \pi/2$.

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(b) **Horizontal Direction**

**Fig. E.4.2: Calculation of Horizontal Angle of Normal**

One can see from Fig. E.4.2 that the horizontal angle is equal to $\theta$.

In order to calculate the various other values in Fig. E.4.2:

\[
\text{SIDE A} = r \cos \theta \quad (E.4-1)
\]

\[
\text{SIDE B} = \sqrt{r^2 - (\text{SIDE A})^2} \quad (E.4-2)
\]

\[
\text{SIDE C} = R - \text{SIDE A} \quad (E.4-3)
\]
(c) Accounting for Both Vertical and Horizontal Displacement

Fig. E.4.3: Pathlength (ρ) from Point Source to Bell Element

The length of the UV path form point source to the centre of the bell element's surface area (ρ) may be simply calculated by means of Pythagorus' theorem, since the co-ordinates of both points are known.

Fig. E.4.4: Calculation of the Length of the Side 'Normal'
Fig. E.4.4 shows how the length of the path made by the true normal to the bell element surface form the centre of that surface to the point where the \( r \) co-ordinate of the line is equal to that of the point source is calculated.

The following must be calculated:

\[
\text{SIDE E} = \text{SIDEC}.\tan\theta \\
\text{SIDE D} = \text{SIDEC}/\cos\theta \\
\text{HGHT (height)} = \text{SIDEC}.\tan(\pi/2 - \psi) \\
\text{NORMAL} = \sqrt{\left((\text{HGHT})^2 + (\text{SIDE D})^2\right)}
\]

Fig. E.4.5: Calculation of the Length of 'Side G'

\[
\text{SIDE G} = \sqrt{\left((\text{ABS}(z-1) + \text{HGHT})^2 + (\text{SIDE F})^2\right)}
\]
We now know the lengths of three lines (the path length, $\rho$; the length of the normal to the bell element surface from there to the point at which its $r$ co-ordinate is equal to that of the point source, $\text{NORMAL}$; and the line joining the point source to the end of $\text{NORMAL}$, $\text{SIDEG}$) which join to form a triangle. By the application of the half angle theorem for $\tan \lambda$, $\lambda$ may be calculated.

Note that provision is made in `belldose.bas` for situations where the vertical distance of the union of the lines 'Side G' and 'Normal' from the point source is not equal to the magnitude of $(z-l)$ minus 'Hght' (i.e. where the bell element is above the point source, but the normal points downward; or the point source is above the bell element, but the normal points upward).
REM
REM  BELLDOSE.BAS
REM
REM  Model parameters
REM
$DYNAMIC
DIM RAD(1:1000)
DIM XCO(1:1000)
DIM PSI(1:1000)
DIM THK(1:1000)
DIM VEL(1:1000)
DIM IBL1(1:1000)
DIM IBL2#(1:1000)
DIM ISR1(1:1000)
DIM ISR2(1:1000)
DIM THETA(1:1000)
REM
ZF=.43
TN=7.2E-2
QV=3E-4
POB=0
PIB=1
DEN=1000
QM=QV*DEN
RP=0.0381
V0=0.55
REM
G=9.81
PI=3.141592654
REM
REM Set up groups
REM
A=2*PI*(POB-PIB)/(QM*V0)
B=4*PI*TN/(QM*V0)
GA=G/V0^2
REM
REM C=1
AL=0.01
REM
REM Set up initial position
REM
RO=RP
Z0=0
D0=0
REM
RAD(1)=R0
XCO(1)=Z0
PSI(1)=D0
REM
V=SQR(V0^2+2*G*Z0)
H=QV/(2*PI*R0*V)
REM
THK(1)=H
VEL(1)=V
REM
R=R0
Z=Z0
D=D0
REM
VB=0
RT=0
REM
RMIN=0
RMAX=0
ZMIN=0
ZMAX=0
REM
REM Calculate RC
REM
100 E=SQR(1+2*GA*Z)
RC=(E-B*R) / (B*SIN(O)+A*R+GA*COS(O)/E)
REM
PHI=AL*0.5/RC
REM
R1=R+2*RC*SIN(PHI)*COS(D+PHI)
Z1=Z+2*RC*SIN(PHI)*SIN(D+PHI)
D1=D+2*PHI
REM
C=C+1
REM
RAD(C)=R1
XCO(C)=Z1
PSI(C)=D1
REM
V=SQR(V0^2+2*G*Z1)
H=QV/(2*PI*R1*V)
REM
THK(C)=H
VEL(C)=V
REM
VB=VB+RC*H
RT=RT+1/V
REM
R=R1
D=D1
Z=Z1
REM
IF Z<ZMIN THEN ZMIN=Z
IF Z>ZMAX THEN ZMAX=Z
IF R<RMIN THEN RMIN=R
IF R>RMAX THEN RMAX=R
REM
IF (Z<ZF) AND (C<200) THEN
  GOTO 100
END IF
REM
N=1
TH0=0
REM
THETA(1)=TH0
REM
TH=TH0
REM
200 TH1=TH+0.017453292
REM
N=N+1
REM
THETA(N)=TH1
REM
TH=TH1
REM
IF (N<360) THEN
  GOTO 200
END IF
REM
VB=VB*2*AL*PI
RT=RT*AL
SCREEN 12
CLS
PRINT
PRINT "ZMIN=",ZMIN
PRINT
PRINT "ZMAX=",ZMAX
PRINT
PRINT "RMIN=",RMIN
PRINT
PRINT "RMAX=",RMAX
PRINT
PRINT "NUMBER OF POINTS IN BELL=",C
INPUT Z9
CLS
REM Display bell profile graphically
REM
SCREEN 12
CLS
REM Calculate scaling factors
REM
ZSC=430/(ZMAX-ZMIN)
RSC=590/(RMAX-RMIN)
REM Choose the smallest of the 2 factors and set the other equal to it so that r and z distances
REM are shown equally on the graph
REM
IF ZSC>RSC THEN
  ZSC=RSC
ELSE
  RSC=ZSC
END IF
REM Plot axes
REM
Y1=-ZMIN*ZSC+20
LINE (20,Y1) - (639,Y1)
LINE - STEP (-12,-8)
PSET (639,Y1)
REM

LINE - STEP (-12,8)

REM

X1=-RMIN*RSC+20
LINE (X1,20) - (X1,479)
LINE - STEP (-8,-12)
PSET (X1,479)
LINE - STEP (8,-12)

REM

 Plot points
REM
REM

X1=(RAD(1)-RMIN)*RSC+20
Y1=(XCO(1)-ZMIN)*ZSC+20
PSET (X1,Y1)

REM

FOR G1=2 TO C
   X1=(RAD(G1)-RMIN)*RSC+20
   Y1=(XCO(G1)-ZMIN)*ZSC+20
   LINE - (X1,Y1)
NEXT

REM

999 S$=INKEY$
   IF S$="" THEN GOTO 999

REM

REM Calculate UV intensity at the bell surface
REM
REM Lamp parameters
REM
NC=3
NL=1
NZ=10
REM
NP=2*NC*NL*NZ
REM
S=18.1
RL=7.5E-3
LL=0.37
LS=0.06
REM
SP=S/NP
REM
CLS
REM Set absorption coefficient for the bell liquid (base e), per metre.
REM
MU=46.52
REM
REM Set number of internal lamps, i.e. 0 or 1.
REM
NLPIN=1
REM
FOR J1=1 TO C-1
REM
   ISUM=0
   I0=0
REM
FOR J=1 TO NZ
  FOR K=1 TO NC
    FOR M=1 TO 2*NL
      REM Calculate the point source co-ordinates
      T=(M-1)*PI/NL
      R=SQR((K-0.5)*RL^2/NC)
      L=(J-0.5)*LL/NZ+LS
      REM Calculate the square of the distance between the point
      REM source and the bell element
      REM Bell element (r,1) co-ordinates are RAD(J1) and XCO(J1)
      REM Theta set to 0.
      RHOS=(R*SIN(T)-RAD(J1)*SIN(0))^2
      RHOS=RHOS+(R*COS(T)-RAD(J1)*COS(0))^2
      RHOS=RHOS+(L-XCO(J1))^2
      REM Calculate the angles for determining the view factor for the
      REM bell element.
      ZET=ATN(RAD(J1)/ABS(XCO(J1)-L))
      LAM=ZET-PSI(J1)
      REM Calculate path length of UV through the bell element

PL = THK(J1)/COS(LAM)
REM
REM Calculate reduction in intensity due to absorption in the bell
REM
NT = 1
IRF = 0
FOR N1 = 1 TO NT
   IRF = IRF + EXP(-MU*(N1-0.5)*PL/NT)
NEXT
IRF = IRF/NT
REM
REM Calculate intensity contribution from point source to bell element
REM
ISUM = ISUM + COS(LAM)*IRF/RHOS
IO = IO + COS(LAM)/RHOS
REM
NEXT M
NEXT K
NEXT J
REM
I = ISUM*SP/(4*PI)
IO = IO*SP/(4*PI)
REM
IBL1(J1) = I
ISR1(J1) = IO
NEXT J1
REM
REM Calculate fluences
F1=0
F01=0

FOR J1=1 TO C-1
    IF NLPIN>0 THEN F1=F1+IBL1(J1)*AL/VEL(J1) ELSE F1=F1+0
    IF NLPIN>0 THEN F01=F01+ISR1(J1)*AL/VEL(J1) ELSE F01=F01+0
NEXT

Set the number of lamps on outside of bell

NLPOUT=16

FOR J1=2 TO C-1

   ISUM#=0
   I0=0

   OPP=SQR(((0.1725)^2)-((RAD(J1))^2))
   CRIT=ATN(OPP/(RAD(J1)))
   DCRIT=CRIT/0.017453292
   CRITNO=CEIL(DCRIT)
   PRINT "CRITNO = ":CRITNO

Calculate the point source co-ordinates

FOR X=1 TO CRITNO
    FOR J=1 TO NZ
FOR K=1 TO NC
    FOR M=1 TO 2*NL
        T=(M-1)*(2*PI)
        R=(SQR((K-0.5)*RL^2/NC)) + 0.1725
        L=(J-0.5)*LL/NZ+LS
    REM
    REM Calculate the square of the distance between the point source and the bell element
    REM
    REM Bell element (r,l,t) co-ordinates are RAD(J1), XCO(J1), THETA(X)
    REM
    RHOS=((R*SIN(T)-((RAD(J1))*(SIN(THETA(X)))))^2
    RHOS=RHOS+((R*COS(T)-((RAD(J1))*(COS(THETA(X)))))^2
    RHOS=RHOS+(L-(XCO(J1)))^2
    RHO=SQR(RHOS)
    REM
    REM Calculate the angles for determining the view factor for the bell element.
    REM
    SIDE A=(RAD(J1))*(COS(THETA(X)))
    SIDE B=SQR((RAD(J1))^2-SIDE A^2)
    SIDE C=R-SIDE A
    IF (PSI(J1))>(PI/2) THEN ANGA=(PSI(J1)-(PI/2) ELSE ANGA=(PI/2)-(PSI(J1))
    HGHT=(TAN(ANGA)*SIDE C
    SID E D=SIDE C/(COS(TH ETA(X)))
    NORM=SQR((S IDED)^2+(HGHT)^2)
    SIDE E=SIDE D*TAN(TH ETA(X))
    SIDE F=SIDE E+SIDE B
    IF PSI(J1)<(PI/2) THEN ZZ=XCO(J1)-HGHT ELSE ZZ=XCO(J1)+HGHT
    SIDE G=SQR(((ZZ-L)^2+SIDE F)^2)
S = (NORM + SIDEG + RHO) / 2
HTLAM = SQRT((S-RHO)*(S-NORM))/(S*(S-SIDEG))
HLAM = ATN(HTLAM)
LAM = 2*HLAM

REM Calculate path length of UV through the bell element
REM
REM IF (LAM<(PI/2)) AND (THK(J1)>0) THEN PL = (THK(J1))/COS(LAM) ELSE PL = 0

REM Calculate reduction in intensity due to absorption in the bell
REM
NT = 1
IRF# = 0
FOR N1 = 1 TO NT
    BL = (MU*(N1-0.5)*PL/NT)
    IF (BL>0) AND (BL<700) THEN IRF# = IRF# + EXP(-BL) ELSE IRF# = IRF# + 0
NEXT
IRF# = IRF# / NT

REM Calculate intensity contribution from point source to bell element
REM
IF LAM<(PI/2) THEN ISUM# = ISUM# + ((COS(LAM)) * IRF#) / RHOS ELSE ISUM# = ISUM# + 0
IF LAM<(PI/2) THEN IO = IO + ((COS(LAM)) / RHOS) ELSE IO = IO + 0

REM
NEXT M
NEXT K
NEXT J
NEXT X
REM
ISUM#=ISUM#/CRITNO
I0=I0/CRITNO
REM
FACT=(2*CRITNO*NLPOUT)/360
REM
I#=(ISUM#*SP*FACT)/(4*PI)
I0=(I0*SP*FACT)/(4*PI)
REM
IBL2#(J1)=I#
ISR2(J1)=I0
NEXT J1
REM
REM Calculate doses
REM
F2#=0
F02=0
REM
FOR J1=2 TO C-1
   IF NLPOUT>0 THEN F2#=F2#+((IBL2#(J1))*(AL/(VEL(J1)))) ELSE F2#=F2#+0
   IF NLPOUT>0 THEN F02=F02+((ISR2(J1))*(AL/(VEL(J1)))) ELSE F02=F02+0
NEXT
REM
ATH=THK(1)
FOR V=2 TO C
   ATH=ATH+THK(V)
NEXT
REM
HAVE=ATH/C

REM Display results

CLS
PRINT
PRINT "BELL VOLUME = ";VB*1000000 "ML"
PRINT
PRINT "AVERAGE DOSE IN BELL = ";(F1+F2)/10 "MW-S/CM^2"
PRINT
PRINT "AVERAGE DOSE AT INSIDE SURFACE OF BELL = ";F01/10 "MW-S/CM^2"
PRINT
PRINT "AVERAGE DOSE AT OUTSIDE SURFACE OF BELL = ";F02/10 "MW-S/CM^2"
PRINT
PRINT "FRACTIONAL REDUCTION IN UV INTENSITY IN THE BELL = ";USING "####.#####";1-
(((F1+F2)/(F01+F02))
PRINT
PRINT "AVERAGE RESIDENCE TIME IN THE BELL = ";RT "S"
PRINT
PRINT "AVERAGE INTENSITY IN THE BELL = ";(F1+F2)/(10*RT) "MW/CM^2"
PRINT
PRINT "AVERAGE (MEDIAN) THICKNESS = ";HAVE*1000 "MM"
PRINT

REM
END
APPENDIX E.6

Program Listing for belldose.bas

DIM RAD, XCO, PSI, THK, VEL, IBL1, IBL2#, ISR1, ISR2, THETA = Dynamic arrays for calculated bell radius, z co-ordinate, the angle ψ, bell thickness, liquid velocity, intensity within the bell element (central lamp), intensity within the bell element (outside lamps), intensity at the element surface (central lamp), intensity at the element surface (outside lamps) and θ co-ordinate (in 1° steps) values respectively.

ZF = Distance fallen from bell nozzle to base of contactor, m.

TN = Surface tension, N.m⁻¹.

QV = Volumetric flow rate, m³.s⁻¹.

POB = Pressure outside bell, Pa.

PIB = Pressure inside bell, Pa.

DEN = Density, kg.m⁻³.

QM = Mass flow, kg.s⁻¹.

RP = Radius of plate at end of nozzle, m.

V0 = Liquid velocity at nozzle, m.s⁻¹.

G = Acceleration due to gravity, m.s⁻².
\[ \pi = \pi, \ 3.141592654. \]

\[ A = \alpha, \text{ from Lance and Perry model, see Appendix E.1.} \]

\[ B = \beta, \text{ from Lance and Perry model, see Appendix E.1.} \]

\[ GA = \gamma, \text{ from Lance and Perry model, see Appendix E.1.} \]

\[ AL = \text{Arc length, m.} \]

\[ V = \text{Liquid velocity, m.s}^{-1}. \]

\[ H = \text{Bell thickness at any point, m.} \]

\[ VB = \text{Volume of the bell, m}^3. \]

\[ \text{RMIN} = \text{Minimum radius of bell, m.} \]

\[ \text{RMAX} = \text{Maximum radius of bell, m.} \]

\[ \text{ZMIN} = \text{Minimum z co-ordinate of bell, m.} \]

\[ \text{ZMAX} = \text{Maximum z co-ordinate of bell, m.} \]

\[ E = e, \text{ from Lance and perry model, see Appendix E.1.} \]

\[ \text{RC} = \text{Radius of curvature of bell profile at any point, m.} \]

\[ \text{PHI} = \phi, \text{ Angle from Lance and Perry model, see Fig. E.1.2.} \]

\[ \text{NC} = \text{Number of circles into which UV source is divided.} \]
NL = Number of lines by which UV source is divided.

NZ = Number of zones into which UV source is divided (lengthways).

NP = Number of point sources into which UV source is nominally divided.

S = Power of UV source, W.

RL = Radius of UV source, m.

LL = Length of emitting portion of UV source (i.e. not including end caps), m.

LS = z co-ordinate of highest end of UV source, m.

SP = Power of a single point source, W.

MU = Napieran absorption coefficient of treated liquid, m\(^{-1}\).

NLPIN = Number of lamps at the centre of the bell (i.e. 0 or 1).

T = Theta co-ordinate of point source.

R = r co-ordinate of point source.

L = z co-ordinate of point source.

RHOS = \(\rho^2\), square of the distance from point source to surface of bell element.

ZET = \(\zeta\), see Fig. E.2.1.
LAM = \( \lambda \), view factor angle.

PL = Path length of light through bell element, m.

NT = Number of points along path length at which intensity reduction due to absorption is calculated.

IRF = Intensity reduction factor.

I = Intensity in bell element (inside lamp), W.m\(^{-2}\).

I0 = Intensity at surface of bell element (inside and outside lamps), W.m\(^{-2}\).

F1 = Dose (fluence) in bell element (inside lamp), J.m\(^{-2}\).

F01 = Dose (fluence) at inside surface of bell (inside lamp), J.m\(^{-2}\).

NLPOUT = Number of lamps outside bell (i.e. 0 or an even number).

OPP = See Fig. E.3.1.

CRIT = Critical angle, see Fig. E.3.1.

DCRIT = Critical angle expressed in degree measure.

CRITNO = Critical number (i.e. the number of 1° steps around the circumference of the bell for which intensities and doses are calculated.

RHO = \( \rho \), distance from point source to surface of bell element, m.

SIDEA = see Appendix E.4.
SIDEB = see Appendix E.4.

SIDEC = see Appendix E.4.

ANGA = Angle A, the vertical angle of the normal, see Fig. E.4.1.

HGHT = see Appendix E.4.

SIDED = see Appendix E.4.

NORM = see Appendix E.4.

SIDEE = see Appendix E.4.

SIDEF = see Appendix E.4.

ZZ = z co-ordinate of the point along the normal to a bell element at which the r co-ordinate is equal to that of the point source, see Fig. E.4.5.

SIDEG = see Appendix E.4.

HTLAM = The tangent the angle equal to half of $\lambda$, from the half tangent formula.

BL = MU*PL

FACT = Factor by which the dose and intensity for the externally-irradiated bell sector are multiplied to account for overlap of irradiation by adjacent lamps.
I# = Intensity in bell element (outside lamps), W.m\(^2\).

F2# = Dose (fluence) in bell element (outside lamps), J.m\(^2\).

F02 = Dose (fluence) at outside surface of bell element (outside lamps), J.m\(^2\).

HAVE = Average, or median thickness of the bell sheet, m.
APPENDIX F.1

Data from Salt Tracer Hydraulic Studies

Fig. F.1.1: Chart Plot from Salt Tracer Experiment

Points selected from the rough plots were plotted on a graphics package (MicroCal Origin 2.8, MicroCal Inc., MA, USA) to produce smoothed peaks (right hand side of Fig. F.1.1), and polynomial expressions were fitted to the upward and downward parts of the peaks. By integration of the polynomial expressions and using a process of iteration, it was possible to work out the time taken for 10% and 90% of the salt to pass through the experimental tracer set-up. These quantities are known as \( t_{10} \) and \( t_{90} \) respectively. Tables F.1.1 and F.1.2 show \( t_{10} \) and \( t_{90} \), \( t_p \) (the time taken for the peak to be reached, determined by direct measurement), and the quantity \( t_{90}/t_{10} \) (known as the Morrill dispersion index). The Morrill dispersion index will equal unity when perfect plug flow pertains, and it has been stated (Thampi, 1990) that for plug flow conditions to occur its value should be less than 2.0. The calculated values for \( t_{90}/t_{10} \) 1.8 and 2.1 respectively, indicate that flow conditions are on the border of the plug flow region.
Table F.1.1: Hydraulic Quantities for a 600 L/hr. Bell

<table>
<thead>
<tr>
<th></th>
<th>t_n</th>
<th>t_10</th>
<th>t_90</th>
<th>t_90/t_10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.3</td>
<td>10.2</td>
<td>16.6</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>23.7</td>
<td>15.4</td>
<td>26.8</td>
<td>1.7</td>
</tr>
<tr>
<td>3</td>
<td>27.8</td>
<td>13.8</td>
<td>28.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Ave.</td>
<td>22.6</td>
<td>13.1</td>
<td>23.9</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table F.1.2: Hydraulic Quantities for a 700 L/hr. Bell

<table>
<thead>
<tr>
<th></th>
<th>t_n</th>
<th>t_10</th>
<th>t_90</th>
<th>t_90/t_10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.6</td>
<td>13.8</td>
<td>29.5</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>19.2</td>
<td>11.1</td>
<td>20.8</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>16.4</td>
<td>8.4</td>
<td>19.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Ave.</td>
<td>19.7</td>
<td>11.1</td>
<td>23.1</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Inactivation Model Program, inact.bas

REM INACT.BAS
REM
NO=4.33E6
REM
NO is the initial count for the culture (CFU/ml).
REM
I=60
REM
I is the average intensity in the contactor (mW/cm^2)
REM
K=0.083
REM
K is the inactivation constant (cm^2.mW^-1.s^-1)
REM
T=0.275
REM
T is the retention time in seconds.
REM
TN=3
REM
TN is the threshold number
REM
SGR=0.093
REM
SGR is the specific growth rate (h^-1)
REM
TVOL=1000
REM
TVOL is the total volume, in litres
REM
LPRINT "TOTAL VOLUME = " ;TVOL "LITRES"
REM
VFR=1200
REM
VFR is the volumetric flow rate, in litres per hour
REM
CT=TVOL/VFR
CT1=FIX(CT)
CT2=CT-CT1
CT2=CT2*60
LPRINT "CIRCULATION OR VOLUME PASS TIME = " ;CT1 "HRS" ;CT2 "MINS"
REM
CT is the circulation time, in hrs.
REM
TT=247
REM
TT = total experiment time, hrs.
REM
LPRINT "EXPERIMENT TIME = " ;TT "HRS"
REM
NP= TT/CT
NP=CEIL(NP)
REM
NP is the number of passes in the experiment.
REM
LPRINT "TIME = 0"
LPRINT "N0 = ";N0
N=N0
LPRINT " 
FOR X=1 TO NP
LPRINT "PASS NO. = ";X
N=N*(1-((1+1/(K*1*T)))^TN))
IF N<1 THEN N=0 ELSE N=N
TIM1=(X-1)*CT+(T/360)
LPRINT "TIME = ";TIM1 "HRS"
LPRINT "POP. AFTER PASS = ";N
LPRINT "N/N0 = ";N/N0
PG=N*SGR*CT
N=N+PG
IF N>N0 THEN N=N0 ELSE N=N
TIM2=CT*X
LPRINT "TIME = ";TIM2 "HRS"
LPRINT "POP. AFTER GROWTH = ";N
LPRINT "N/N0 = ";N/N0
LPRINT " 
NEXT X
STOP
END
REFERENCES


Brackett, F.P., and Forbes, G.S., (1933). Actinometry with Uranyl Oxalate at \( \lambda \lambda 278, 253 \) and 208 m\( \mu \), Including a Comparison of Periodically Intermittent and Continuous Radiation. Journal of the American Chemical Society, 55, 4459-4466.


Watson, H.E., (1908). A Note on the Variation of the Rate of Disinfection with the Change in the Concentration of Disinfectant. Journal of Hygiene, 8, 536.


