EFFECT OF TRANSMITTANCE AND SUSPENDED SOLIDS ON THE EFFICACY OF UV DISINFECTION OF BACTERIAL CONTAMINANTS IN WATER

by

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ERRATA

Additional information

- page 18 line 23 **NTU** refers to nephelometer transmission units.
- page 20 line 27 **MPN** refers to a most probable number estimate of numbers of bacteria.
- section 3.4 page 42. Water samples were not filtered to remove Celite. Removal of Celite by centrifugation could have resulted in loss of bacteria from the suspending water and underestimation of numbers of surviving (viable) bacteria.
- section 4.5 page 75 line 12. *following* "... initial cell numbers" insert "(*see* page 54 also Table 4.4)"
- Appendix F page 96. *following* the summary table insert "The summary table shows clearly that the water flow in the UV unit is turbulent."

Corrections

- page2 line 11,12; page 6 line 6; page 22 line 15; page 41 line 5 and 6; page 62 Table 4.5 change "absorption" to "absorbtion".
- page 4 line 15 change "... of the efficacy of disinfection systems is the total viable faecal numbers of coliform bacteria." to "... of the efficacy of disinfection is the total number of viable faecal coliforms in the system."
- page 8 Figure 2.3 change "thymidine" to "thymine"
- page 18 second dot point change ".. afford protection ..." to "... are protected ..."
- page 22 line 2 change "... clumps larger than 70 μm caused a major obstacle to inactivating more than ..." to "... clumps larger than 70 μm restricted inactivation to ..."
- page 23 line 22 *following* "... 2 to nearly 8" insert "... 2 to nearly 8 $\log_{10}$"
- page 51 last sentence *following* "... from 0.074% to 0.26% ..." should read "... from 0.074 to 0.026% ..."

Typographical errors

- page 2 second dot point no italics for "and"
- page 2 third dot point no italics for "transmittance"
- page 15 second sentence delete "that"
- page 16, 20, 24 "Schieble *et al.* 1986" should read "Schieble 1986"
- page 18 second dot point no italics for "clumping"
- page 41 line 10 "Mann, 1992" should read "Mann & Cramer, 1992"
- page 59 para. 2 line 4 "Qualls *et al.* 1985" should read "Qualls *et al.* 1989" and "Job *et al.* 1995" should read "Job & Realey"
- page 111 "and" and not "aand"

Order of References

Sabotka 1992 should be listed before Sobottka 1993, *also* Cairns 1993 before Cairns 1995

Additional Reference

page 37 Stanier *et al* 1972: Stainer, R. Y., Doudoroff, M. and Adelberg, E. A. 1972. *General* Microbiology, 3rd Edn., The Macmillan Press Ltd., London.

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Date: 17 March 1999

SUMMARY

Irradiation with ultraviolet light is an effective means for disinfection of contaminating bacteria in potable water and waste-water. However high levels of turbidity and suspended solids (SS) can limit UV efficacy. Little quantitative data however are available. To obtain robust and quantitative data on the influence of UV absorption and SS on UV disinfection an experimental study using commercial disinfection technology was undertaken. The acquisition of data is justified by an increased confidence in application and understanding and as a necessary step to process optimisation.

A commercial disinfection unit (UV-LC5™ from Ultraviolet Technology of Australasia P/L) was operated with a range of feed water low rates (1 - 4 L min^{-1}) and which contained either *Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* as selected test micro-organisms. *E. coli* was selected because this is found in sewage or water contaminated by faecal material, is used as an indicator for presence of other enteric pathogens and it should be absent in potable water. *P. aeruginosa* was used as a test bacterium primarily because it has DNA comprising relatively high molar ratios of guanine (G) and cytosine (C) and is therefore more resistant to inactivation by UV light than *E. coli.* UV dosage (6,500 - 25,000 $\mu W.s.cm^{-2}$) was altered by controlling the flow rate of feed water into the disinfection unit. The transmittance of feed water (at 254 nm) was adjusted by addition of a UV absorbing agent (International Roast ™ coffee-powder), or by adjusting turbidity using diatomaceous earth as a suspended solid (SS) (Celite 503™ - 0.01 to 0.1 g L^{-1}, median particle size of 23 μm).

Reductions in the number of viable bacteria of between 3 log_{10} and 5 log_{10} were obtained. Survival of the test micro-organisms was greatest at the highest flow rates used and inversely proportional to UV transmittance of the feed tank water, irrespective of the method by which transmittance was adjusted. However, at equivalent transmittance, Celite provided greater protection against disinfection than addition of a UV absorbing agent. In both dark and light storage post irradiation, the re-growth and repair rate of *E. coli* was greater than for *P. aeruginosa*. Following a six (6) day storage the number of *E. coli* reached nearly 25% of the initial number in un-irradiated water. This work highlights the impact of water quality on the use of small scale UV disinfection units for preparation of potable water, where operating parameters should be based on a knowledge of the presence of soluble UV absorbing agents and of SS.

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Mr. Wayne Bradford for his technical assistance.

I hope that the results of my efforts justify the expectations and confidence of the people concerned, and the interest, help, and encouragement of my family, friends and colleagues.

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APPENDICES

CHAPTER 1 - INTRODUCTION

Disinfection[1] is the *inactivation (destruction)* of micro-organisms capable of causing diseases. Disinfection is an essential procedure for provision of both drinking water (water supply) and waste-water treatment applications. Since chlorination was introduced, initially in Austria in 1847, the use of chlorine for disinfection of water has become the most common worldwide approach - although a number of other methods including ultraviolet light irradiation and osonation are used (Anon. 1982). However, there are a number of important drawbacks with chlorine gas: it is a hazardous material, requires sophisticated application techniques and, may be retained in residuals in water supplies as organic matter. Chlorination efficacy as a disinfectant is greatly affected by changes in pH and temperature of water (Martin & Martin, 1991).

One alternative to chlorination is the use of *ultraviolet* (UV) radiation. UV inactivation of contaminating micro-organisms in water has a number of advantages including the efficient inactivation of both bacteria and viruses, prevention of formation of harmful by-product residuals (unlike chlorination), absence of taste and odour problems, simplicity of handling and storing and low energy and small space requirements (Bitton, 1994).

Water and waste-water that has been successfully treated using UV irradiation include :

- residential and municipal drinking water including surface and ground waters
- that from industrial processes such as food and beverage processing cooling water and aquaculture
- municipal treatments.

Additionally, UV disinfection has been studied for application to storm flows and may provide a future option in water quality improvement schemes (O'Shea & Field, 1992).

High levels of water turbidity and suspended solids can limit UV efficacy however. Little quantitative data on the influence of these factors has been reported. This lack of quantitative and objective data presently limits the wider application of UV disinfection to

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[1] *see* Appendix A for a definition of important terms used throughout this thesis

provision of potable water and treatment of waste-waters. The acquisition of robust data could be justified by an increased application of UV treatment and as a necessary step to its optimisation.

The principal aims of this study are to obtain objective data on the influence of turbidity, as measured by suspended solids (SS), and ultraviolet (UV) dose on the disinfection of bacterial contaminants in selected water. Specifically, to:

- determine the efficacy of UV irradiation on inactivation of *Escherichia coli* and *Pseudomonas aeruginosa*
- compare the susceptibility of *E. coli and P. aeruginosa* to UV light
- examine the effect of water UV *transmittance* and SS on the efficacy of UV inactivation - and in doing so differentiate between the effect of limiting absorption of UV light only, and; absorption, shielding and flocculation with increasing SS
- determine the potential for bacterial regrowth and repair after exposure to UV irradiation, especially when treated water is stored under different conditions
- devise appropriate inactivation kinetics for UV disinfection of the two test micro-micro-organisms.

E. coli was selected as a test micro-organism because this is found in sewage or water contaminated by faecal material, is used as an indicator for presence of other enteric pathogens and it should be absent in potable water. *P. aeruginosa* was used as a test bacterium primarily because it has DNA comprising relatively high molar ratios of guanine (G) and cytosine (C) and is therefore more resistant to inactivation by UV light than *E. coli.*

Chapter 2 presents a summary analysis of the relevant published literature and elicits the factors that affect the efficacy of UV inactivation, and the method of design of UV systems, including the economics of UV disinfection.

The design of the experimental studies conducted are given in Chapter 3.

In Chapter 4, the relationship between survival of the irradiated test micro-organisms and UV dose, UV transmittance and concentration of suspended solids is presented. The

difference between the effect of absorbing material and suspended solids is highlighted. Comparisons are made between the sensitivity of *E. coli* and *P. aeruginosa* to UV light, and in re-growth and cell repair rate.

Chapter 5 is a summary of the results of this research and conclusions together with suggestions for further work.

CHAPTER 2 - LITERATURE REVIEW

2.1 Introduction

The theory and practice of the disinfection of various bulk water using UV light is well documented. For decades it has been known that efficacy depends on the UV wavelength used. Short wave UV radiation that between 220 nm and 300 nm is an effective germicidal agent. Maximum irreparable damage to cells is caused by exposure to UV radiation at a wavelength of 265 nm (Crandall, 1986). Efficacy is reduced with increased wavelength and becomes non-existent above 300 nm (Bachmann, 1975). Figure 2.1 illustrates this relationship between germicidal effectiveness and UV wavelength.

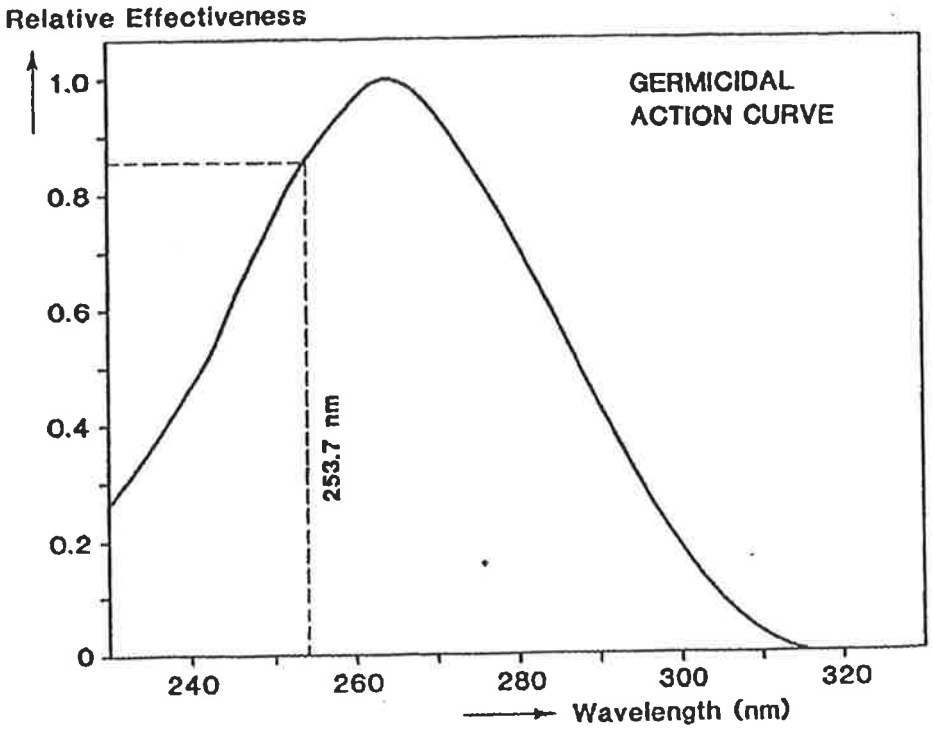
UV radiation is a classed as physical rather than a chemical disinfectant. Unlike chlorination disinfection, no harmful chemical residual or taste or odour problems result. The principal mechanism for inactivation of micro-organisms by UV light is the direct damage to cellular nucleic acid. Oliver *et al.* (1975) found that bacteria were destroyed or rendered inactive by UV light due to photochemical alternation of deoxyribonucleic acid (DNA) in the cell. A widely used performance indicator of the efficacy of disinfection systems is the total viable faecal numbers of coliform bacteria.

2.2 UV disinfection

2.2.1 Sources of UV radiation

There are several sources of UV radiation, however, the most important has been the mercury vapour lamp. Lamps are classified as low or high pressure based on the vapour pressure of the mercury discharge. Low pressure lamps have a pressure between 1.333 x 10^{-2} mbar and 1.333 mbar. High pressure lamps have a vapour pressure of from 1.33.3 bars to 20.26 bar (UVTA *pers. comm.*). Low pressure mercury arcs emits mainly (*c.* 85-90%) at 254 nm, about 7-10% at 185 nm, and the remainder at a wavelength of about 254 nm (Figure 2.2).

Figure 2.1 **The relationship between germicidal effectiveness and UV wavelength**
(Adapted from Meulemans, 1987)



For the purposes of inactivation of bacteria, low pressure mercury arcs are typically used because the main emission spectrum of 254 nm is very close to the wavelength of the highest inactivation efficiency. As can be seen in Figure 2.2, the relative intensity of light emitted from high pressure mercury arcs is spreads more widely over the spectral range than is the case for low pressure units. Furthermore, the amount of both radiated light and self absorption of the UV radiation increases with increasing pressure.

2.2.2 Mechanism of UV damage

UV light (at 260 nm) inactivates by directly damaging nucleic acids. The UV energy absorbed causes dimerization of adjacent thymine on the same DNA strand. This prevents normal DNA replication and effectively results in the death of cells.

UV light penetrates cells and is absorbed by thymine and cytosine. The formation of thymine dimers distorts the DNA and thus interferes with DNA replication and transcription (Cano & Colome, 1986). Damage caused by sub-lethal doses of UV light can be repaired by either light activated or dark repair systems. This is shown schematically in Figure 2.3. The efficiency of these repair mechanisms depends on exposure time, UV intensity and temperature.

Like bacteria, the primary site of damage to viruses is the genome, followed by structural damage to the virus coat (Rodgers *et al.* 1985).

2.2.3 UV disinfection of potable water and waste-water effluents

UV technology has rapidly expanded into the field of potable and waste-water treatment over the past decade. UV radiation is used as an alternative disinfectant for drinking water in over 2000 European communities (Foust, 1988), and; more than 30 large-scale UV systems were built or planned in the 1980s in North America (Qualls & Johnson, 1985).

Figure 2.2. **Emission spectrum of the low pressure mercury arc and the high pressure mercury arc**
(Adapted from Sonntag & Schuchmann, 1992).

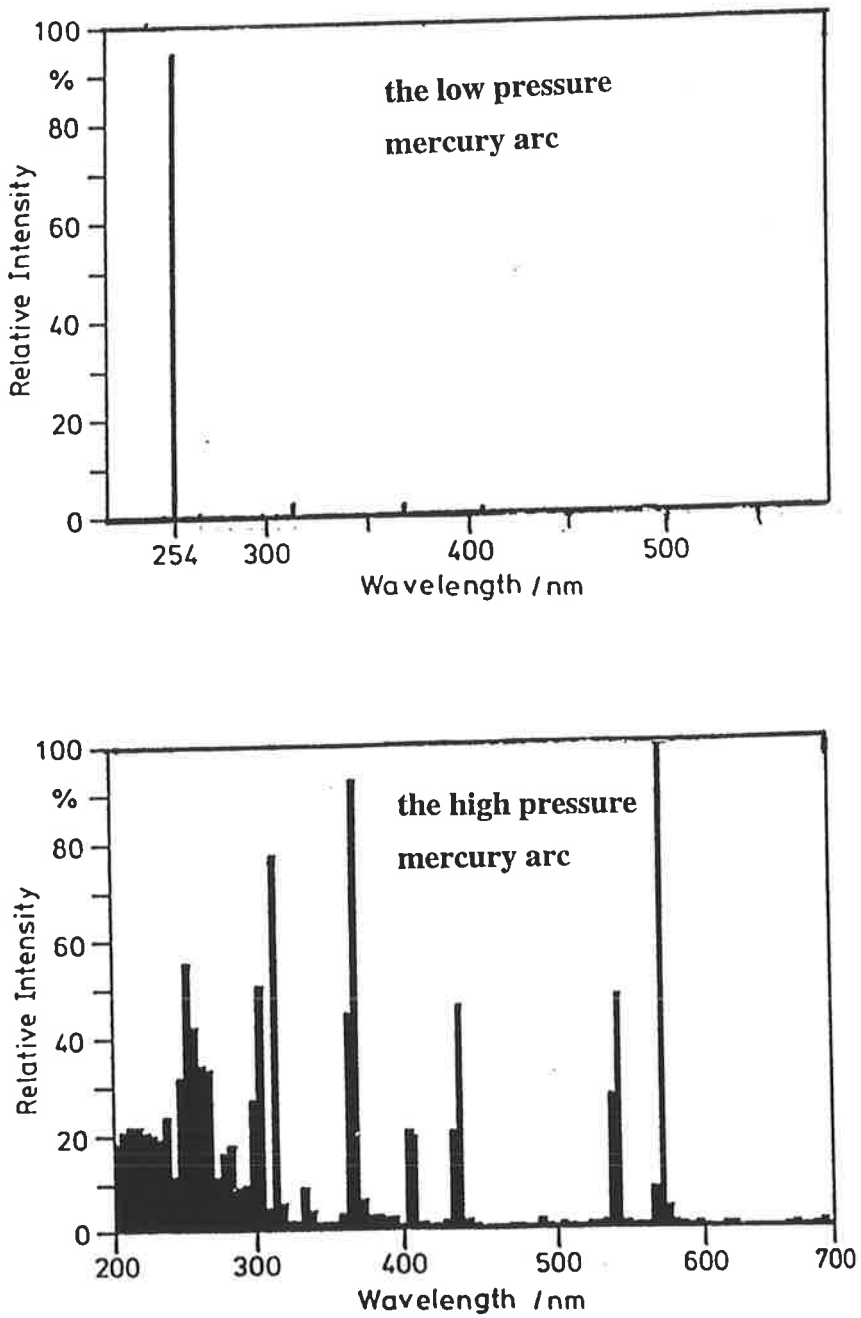


Figure 2.3 **Thymidine dimers distort the DNA molecule and prevent DNA replication and transcription**

(Adapted from Cano & Colome, 1986)

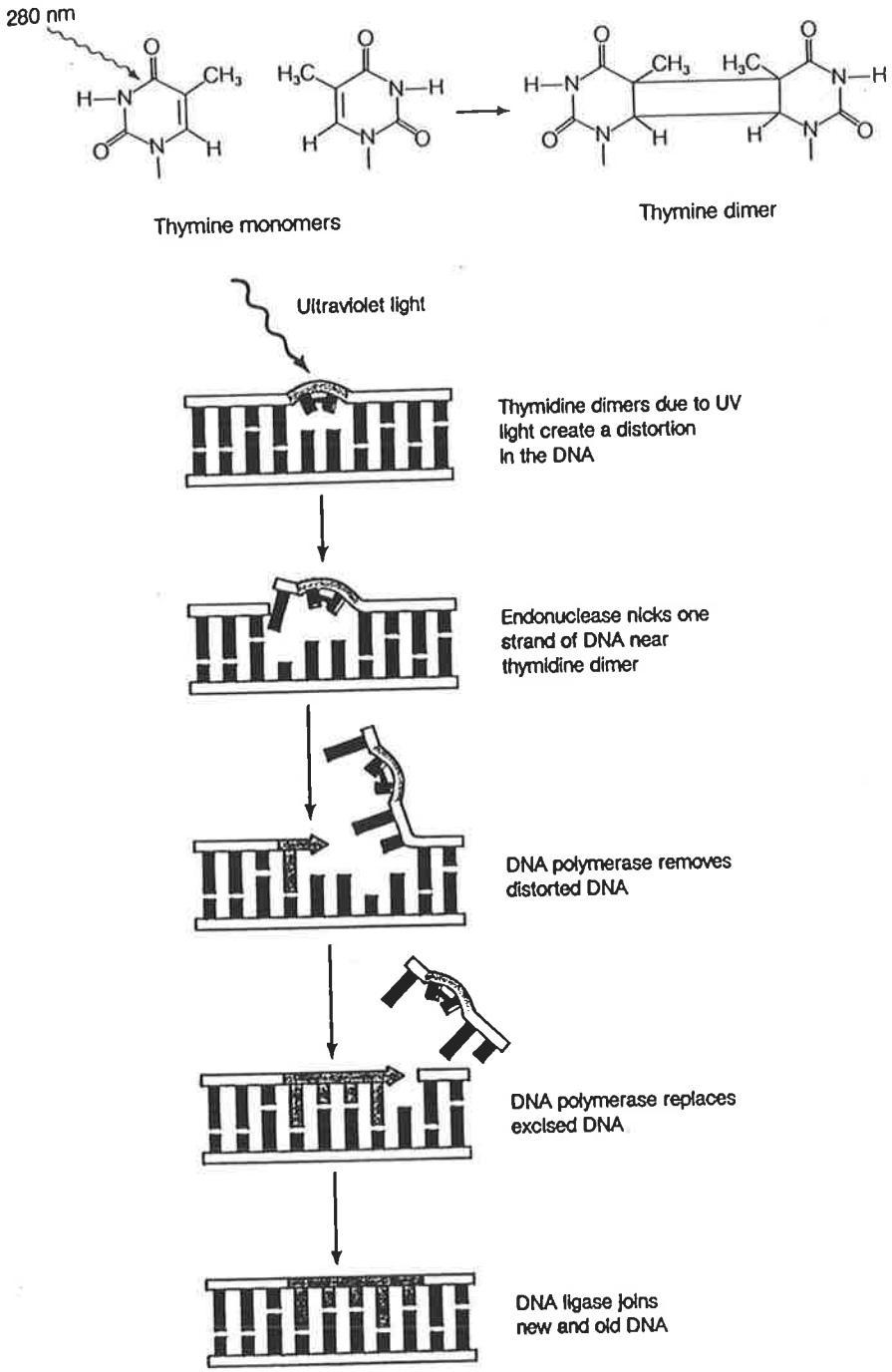


Table 2.1 **AFP* tube ultraviolet disinfection systems inactivate selected water-borne organisms**

(Adapted from Ultraviolet Technology of Australasia P/L., *pers. comm.*)

Waterborne organisms pathogenic to man	Disease	Typical effectiveness
Bacteria		better than
Salmonella typhi	Typhoid fever	99.99%
Salmonella enteritidis	Gastroenteritis	99.99%
Shigella disenteriae	Dysentery	99.99%
Vibrio cholerae	Cholera	99.99%
Enteropathogenic Escherichia coli	Gastroenteritis	99.99%
Leptospira icterohaemorrhagiae	Leptospirosis (Weil's disease)	99.99%
Mycobacterium tuberculois	Tuberculosis	99.99%
Legionella pneumophila	Legionnaires' disease	99.99%
Viruses		
Polio virus	Poliomyelitis	99.99%
Bacteriophage	Gastroenteritis	99.99%

Tested by Medvet Science Pty Ltd, Adelaide, a NATA Registered Laboratory

***Advanced Fluoropolymer Tube (Teflon™ DuPont)**

UV disinfection is increasingly applied to secondary effluents, especially in new municipal water treatment facilities (Wolfe, 1990). Improvements in equipment reliability and the reduction of undesirable by-products continues to increase the popularity of UV technology.

Nevertheless, several problems are associated with the use of UV light inactivation systems. For example, short-circuiting through the UV chambers causes the actual retention times to be greater than the theoretical retention time (Thampi & Sorber, 1987). This problem is exacerbated by the fact that UV dosages cannot be measured reliably and consistently. In addition, *bio-fouling* of the lamp surface can decrease the effective bactericidal intensity. Finally, bacterial repair mechanisms may limit usefulness (Preez & Kfir, 1995; Hengesbath *et al.* 1993). Some of these problems can be resolved. For example, un-perforated metal sheets installed at the inlet and a perforated one at the inlet and outlet, minimise short-circuit flow within the unit (Dizer *et al.* 1993). Other, technical improvements involve development of accurate monitoring devices and controls (Crandall 1986).

UV radiation also plays a major role in the degradation of synthetic compounds in the environment (Crosby, 1972). It is reported that many chemical substances such as phenolic compounds, humic acids, lignin sulphonates, iron, and coloring agents interfere with UV transmission at 254 nm (Huff *et al.* 1965; Yip & Konasewich, 1972). In other studies, application of UV light in combination with ozone or peroxide were examined to assess suitability for removal of organics as an alternative to activated carbon and osmosis. The total organic carbon (TOC) of lime-clarified and -filtered plant water can be successfully reduced to 2 mgL^{-1} - the level present in drinking water (Lauer *et al.* 1991).

2.3 UV disinfection in combination with oxidants

In order to improve the efficiency of UV disinfection, the combination of UV radiation and oxidising agents such as hydrogen peroxide and ozone has been tested. Generation of hydroxyl radicals through UV photolysis of oxidisers is the key principal of UV/oxidation

technologies and are summarised by the following equations (Kirankumar 1993; Prengle, 1983):

$$H_2O_2 + h\nu \rightarrow 2OH\bullet \quad (2.1)$$

$$O_3 + h\nu + H_2O \rightarrow H_2O_2 + O_2 \quad (2.2)$$

$$H_2O_2 + h\nu \rightarrow 2OH\bullet \quad (2.3)$$

or:

$$2O_3 + H_2O_2 \rightarrow 2OH\bullet + 3O_2 \quad (2.4)$$

Crandall (1986) used a UV/H_2O_2 process to treat and control the rate of contamination from both bacteria and non-biological organic materials commonly found in public hot-water spas and hot tubs. Results showed that the efficacy of inactivation of *Bacillus subtilis* by the combined process was over 2000 times greater than with UV alone (Catherine, 1979). A similar response was observed when using UV light as a separate disinfection method for inactivation of bacteria in swimming pool water. A 50% reduction in numbers of viable bacteria was achieved after a three-time water exchange, whilst a strong inactivating effect of UV combined with hydrogen peroxide or ozone resulted in a 400-fold reduction in levels of bacterial toxins (Sobotka, 1992). Thus, the use of UV alone may not be sufficient in large swimming pools even where high flow rates through the UV disinfection unit are maintained. However, the level of hydrogen peroxide used in spa and swimming pool must be controlled at 20 ppm as a minimum with an ideal range of 30-40 ppm for safety to bathers (Crandall, 1986).

2.4 Inactivation of pathogens by UV irradiation

When micro-organisms are subjected to UV light, they are not inactivated at once, but a constant fraction of the present viable number dies in each increment of time (Koller, 1965). The survival ratio is the fraction of the initial number of micro-organisms present at any given time.

The mathematical expression of these facts can be described as:

$$\text{Survival ratio} = \frac{N_t}{N_0} = e^{-kIt} = e^{-kD} \tag{2.5}$$

where: N_o = number of viable bacterial cells initially present; N_t = the number of viable cells surviving at time t; t = the time of exposure (s); I = the intensity ($\mu W.cm^{-2}$); D = UV dose ($\mu W.s.cm^{-2}$); k = inactivation rate coefficient which depends on the type of micro-organisms and wavelength of UV light.

Equation 2.5 can be more widely expressed as:

$$\ln \frac{N}{N_0} = -kIt = -kD \tag{2.6}$$

2.5 Effect of some process factors on efficacy of UV disinfection

Factors which influence the efficacy of UV disinfection can be divided into three groups (Topudurti *et al.* 1993)

- physio-chemical properties of water (pH, turbidity, dissolved organic and inorganics, type of micro-organisms, cyanuric acid level, particle solids, colour etc.)
- UV unit operating parameters, including UV dosage, contact time
- maintenance requirements.

Meulemans (1987) concluded that the emission spectrum of the UV-source, the intensity of the irradiation, exposure time, the UV sensitivity of contaminating micro-organisms, the required survival ratio and the performances of the reactor were important parameters affecting UV disinfection.

Among parameters characterising quality of waste-water, suspended solids and volatile solids BOD_5, coliform bacteria, and faecal streptococci played an important role in the performance of UV disinfection followed by sedimentation. Other pilot and full-scale

studies of UV disinfection of waste-water have shown the UV dose, the hydraulics which determine bactericidal efficacy, and the degree of unit maintenance were also critical factors affecting the performance of a reactor.

The characteristics of the water to be treated also play an important role in ultraviolet disinfection. UV light must be absorbed by micro-organisms to effect disinfection. Thus, anything in the water that absorbs UV radiation, or shields the micro-organisms from the UV light source, would be expected to influence the efficacy of inactivation. Paradoxically, Savolainen (1991) proposed that although suspended solids and UV absorbency appeared to influence numbers of survivors, waste-water parameters did not have any effect on the survival rate of the micro-organisms.

Nevertheless, different approaches to treatment or disinfection should be considered because of significant differences in the microbial quality of water sources (municipal or industrial and surface or ground water, for example). Before disinfection, the quality of representative water samples should be assessed. Yip and Konasewich (1972) have shown that UV transmittance (at 254 nm) was significantly decreased by chemical substances and suspended solids. High-quality effluents, in terms of turbidity and content of suspended solids, can be consistently produced using tertiary granular sand filters without chemical addition (Braunstein *et al.* 1996). Colour and turbidity, on the other hand, are not necessarily reliable indicators of UV transmittance.

Inactivation efficacy can be increased by increasing turbulence (Cortelyou *et al.* 1954). Several studies show that the efficiency of waste-water disinfection processes are often measured by estimating coliform densities (most probable number (MPN)). The accurate measurement of coliform density in secondary effluent, however, is influenced significantly by particle association and *shielding* of coliforms. Hence, suspended solids is one of several factors that significantly affect all disinfection process.

Surprisingly, the influence of water pH and temperature on disinfection efficacy have received little attention because UV efficacy is assumed to be independent of pH and

temperature. Thus these factors have been ignored in most published studies (Gross & Murphy, 1993).

Typically, disinfected effluents must not contain more than 200 coliforms per 100 mL, although in some cases up to 2500 coliform per 100 mL may be permitted (Darby *et al.* 1993). For drinking water, in particular, the two following standards are important and noteworthy (Bitton, 1994):

- colourless, clear, cool as well as perfectly odourless and tasteless
- contain no visible organisms, residues of animals or plants nor any undissolved organic matter.

In Australia, there are no legal microbiological or related standards for drinking water. According to the National Health and Medical Research Council/Australia Water Resources Council (NHMRC/AWRC) guidelines, samples should not contain any faecal coliforms in 100 mL and 95% of scheduled samples should not contain coliforms in 100 mL, although occasionally up to 10 coliforms may be accepted (Bitton, 1994).

For the practical assessment of UV plants, test micro-organisms must have the following characteristics:

- steady inactivation kinetics over a wide dose range
- simple and suitable methods for cultivation
- be non-pathogenic
- possess temporal stability with regard to UV sensitivity.

Staphylococcus aureus bacteriophage A994 was used by Sommer and Cabaj (1993) as a model for pathogenic viruses. Considering data from four different UV reactors, Havelaar *et al.* (1990) found that somatic coliphages were most sensitive to UV radiation, followed by *E. coli* and faecal streptococci. However, coliforms are less resistant to UV inactivation compared with many pathogenic viruses, bacterial and fungal spores and protozoan cysts (Sobsey, 1989). The amount of UV dosage required to inactivate various micro-organisms, in general, increases in the order: bacteria, viruses, protozoan (Table 2.1). Thus, the

absence or inactivation of coliforms does not necessarily mean that the effluent is specific pathogen-free.

Viruses appear to have a much higher resistance to UV light in comparison to bacteria (Qualls *et al.* 1984). Chang *et al.* (1985) demonstrated that viruses are usually 2-5 times more resistant than that faecal bacteria. Based on equal dose of exposure, the level of sensitivity to UV light of various micro-organisms was established in the following sequence: *E. coli* > coliform organisms > poliovirus type 1 > spores of *B. subtilise* (Dizer *et al.* 1993).

Furthermore, gram-negative bacteria (with thin murein or peptidoglycan cell wall layers) generally are easier to inactivate than gram-positive bacteria (which have thick murein cell walls) (Meulemans, 1987). Spores are more difficult to inactivate due to the very thick cortical and envelope layers surrounding the spore cytoplasmic membrane. In comparison with bacteria, algae required a hundred times more energy for inactivation.

Originally, the term UV dose, D ($\mu W.s.cm^{-2}$) was described as the product of average UV light intensity I ($\mu W.cm^{-2}$) and the average exposure time t (s):

$$D = I \times t \tag{2.7}$$

However, in practice the overall process for dimensioning is quite complex and UV dose is affected by many different parameters as shown schematically in Figure 2.4.

UV doses used for inactivation are relatively low compared with the destructive doses of UV used in advanced oxidation technologies. UV light reacts only with molecules which absorb UV, including a large number of microbial molecules such as the sugar-based extracellular polymers. The UV disinfection dose requirements are different for each type of micro-organism and also depends on experimental conditions. This is in agreement with Darby *et al.*(1993) who showed that UV doses for treating waste-water under laboratory conditions were lower than for field conditions (1-16 $MW.s.cm^{-2}$ *cf* 10-37 $MW.s.cm^{-2}$, respectively). Hence, with similar UV doses, greater inactivation occurred under laboratory

conditions as compared to field conditions (based on log survival data). Darby *et al.* also reported that UV doses used in their studies (ranged from 30-239 $MW.s.cm^{-2}$) were much greater than those reported in the general literature. Past research has shown applied UV radiation intensity is restricted in the range 10 $\mu W.cm^{-2}$ to 1 $MW.cm^{-2}$. Presently, the range of dosage of 30 to 35,000 $\mu W.s.cm^{-2}$ has been applied in most commercial water treatment operations. Tobin *et al.* (1983) suggested a minimum dose of 16,000 $\mu W.s.cm^{-2}$ which is about 2.2 times more than the UV dose required to inactivate *E. coli*. To disinfect waste-water when the transmittance is 65% or higher, a UV dose of 16,000 $\mu W.s.cm^{-2}$ was recommended (Trojian Technologies Inc. 1990). *Enchytraeus albidus* was used by Sobotka (1993) as bio-indicator for calculation of UV dose and assessment of UV disinfection efficiency. This research was conducted under laboratory conditions as well as in selected swimming pools and water works. In order to inactivate 50% of the test organisms the required UV dose was estimated to be 9500 $Ws.m^{-2}$.

In accordance with Austria Codex Alimentarius (Sommer & Cabaj, 1993), disinfection of drinking water by UV light, requires a UV dose of at least 30 $MJ.cm^{-2}$ at the point of the greatest distance from the lamp. Based on the results of a previous study (Oliver & Cosgrove, 1975), they noticed that bacterial inactivation depends only on total light dosage at 254 nm and not on light intensity.

The relationship between UV dose and survival of micro-organisms described in much of the literature shows that a more rapid reduction in viable numbers is to be expected at very high UV dose. Therefore, the UV dose used depends on the discharge permitted of resistant micro-organisms (eg. 2.2, 23, 240 or 1,000 coliform per 100 mL) (Loge *et al.* 1996). The survival of the micro-organisms, moreover, was determined as a function of the UV dosage in order to "standardise" the sensitivity of the micro-organisms. On the other hand, Schieble *et al.* (1986) reported that no change was found in the degree of photo-reactivation with the UV dose. This is in agreement with Whitby and Palmeteer (1993), who concluded that the UV transmittance (dose) and suspended solids did not affect the degree of photo-reactivation.

Table 2.2 Summary of typical UV doses required for different type of micro-organisms

Micro-organism	% inactivation	UV dose required ($\mu W.s.cm^{-2}$)	Reference
Giardia lamblia cysts	less than 80	63,000	Wolfe, 1990
Giardia muris cysts	90	82,000	Wolfe, 1990
F-specific RNA coliphage Qß	90	3,600	Kamiko *et al.* 1989
Bacillus spp.	30 CFU/100 mL	30,000	Savolainen, 1991
Viruses	99.9	20,000-30,000	Jakab *et al.* 1982
Airborne viruses	99	1260	Jakab *et al.* 1982

Recent work by Cairns (1995) demonstrated that typical UV dosage for drinking water, ranges from 16 to 40 $MW.s.cm^{-2}$ depending on the application, water quality, and target disinfection. In the case of waste-water treatment, the UV dose was dependent upon the power output of the lamp, the waste-water UV transmittance and the flow rate (Job & Realey, 1995).

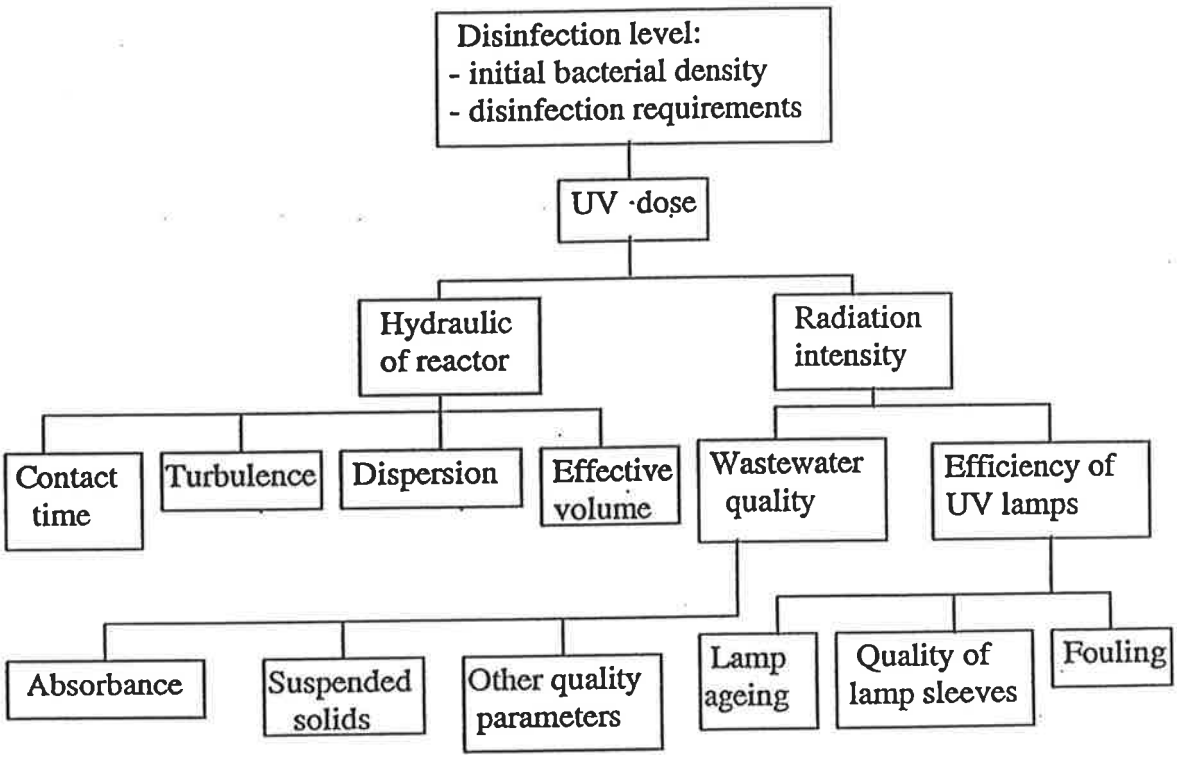
However, because of different biological conditions such as culturing methods for preparing the test micro-organisms or because of technical problems regarding UV irradiation equipment and UV dose measurement, measurements for even of the same species of micro-organisms might show significant differences in UV susceptibility (Table 2.2).

The effect of suspended solids on UV inactivation is threefold (Severin & Suidan,1985):

- *clumping* of micro-organisms skew the kinetic response due to the method by which survival is measured, ie. the plate count method
- micro-organisms which clump together afford protection from UV inactivation due to association with organic particles, a typical limiting factor for UV as well as other disinfectants
- UV light is scattered (*see* Figures. 2.5 and 2.6)

Micro-organisms in water may be embedded in, or otherwise associated with, SS in ways that often interfere with the microbiocidal process. Suspended solids reduce efficiency of UV disinfection, not only by scattering and absorbing radiation, but also shielding them from exposure to UV light. Thus the overall performance of UV disinfection units was reduced with higher suspended solids concentrations, (Job & Realey, 1995). However, suspended solids in the range of 5-50 $mg.L^{-1}$ and turbidity from 0.5-12 NTU had little effect on UV absorbency (Darby *et al.* 1993). For the best results for disinfection to a standard of 100-200 faecal coliform/100 mL, in treatment waste-water, levels of suspended solids should be less than 20 $mg.L^{-1}$ (White *et al.* 1986).

Figure 2.4 **Parameters affecting the UV dose in microbial inactivation** (Adapted from Savolainen, 1991).

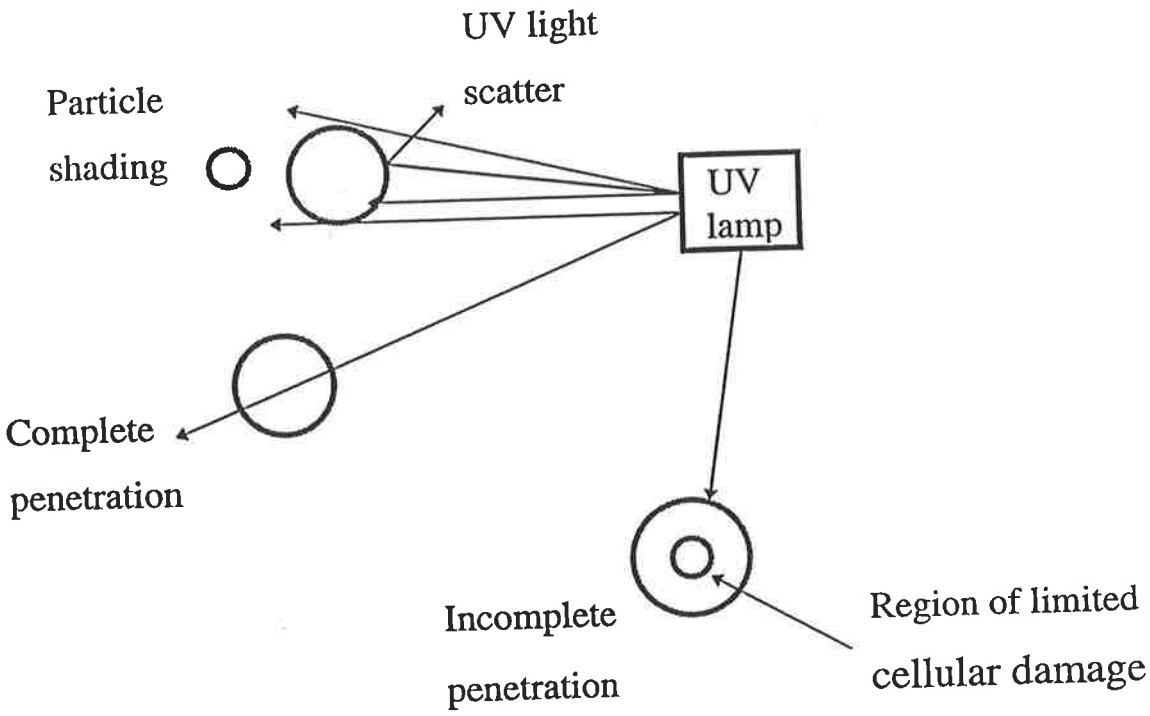


Suspended solids also cause tailing plateaus in the Dose-survival curve. This phenomenon is due to both scattering and incomplete penetration of UV light (Loge *et al,* 1996). Dizer *et al* (1993) estimated that the inactivating action of UV radiation on *Coliphage f2* was considerably lowered from 97% to an unsatisfactory 54% due to influence of suspended particles. Kaolin or activated sludge were added in the water samples as suspended solids. It was found that the inactivation rate did not change even with increasing concentration of kaolin (Kamiko & Ohgaki, 1989). In order to explain these results, they assumed overall absorbency of the sample (A_o) included the absorbency of filtrate (A_f) and the absorbency caused by the suspended solids(A_s). Moreover, for samples containing kaolin the inactivation rate was much greater than in the case of samples containing activated sludge. Therefore, they suggested that it was impossible to predict the inactivation rate of samples containing suspended solids even though the values of A_o, A_f and A_s could be known.

There was no correlation found between suspended solids (5 to 120 $mg.L^{-1}$) and the number of faecal coliforms in the irradiated effluent which contained 10^4 to 10^7 faecal coliforms per 100 mL (Petrasek *et al.* 1980). This indicated that high level of SS probably leads to low UV transmittance or inadequate mixing of the effluent in the shallow irradiation trays. These results were not in agreement with Whitby and Palmateer. (1993) and Shieble *et al.* (1986). Based on data in their investigations, a linear relationship between SS and the surviving faecal coliforms was demonstrated. In addition, they concluded that although there was no correlation between the ratio of the photo-repaired and non photo-repaired faecal coliforms after UV exposure and suspended solids, the photo-reactivation had a significant effect on the level of suspended solids which can be disinfected. It was shown that the difference between the degree of photo-reactivation with the different levels of UV transmittance and SS might be due to the degree of damage to the DNA.

In nature, coliforms are likely to be particle-associated than are the phages (seeded micro-organisms) (Braunstein *et al.* 1996). It is known that the MPN increased from an initial value to a maximum value with increasingly vigorous blending and then reduced again. Qualls *et al.* (1983) concluded that the removal of particles large enough to shadow coliforms exerted dramatic effects on the dose-survival curves. The coliforms which have

Figure 2.5 **Effect of suspended solids on UV disinfection**
(Adapted from Loge *et al.* 1996)



size of greater than 8μm were extremely resistant to UV. Further, the clumps larger than 70 μm diameter caused a major obstacle to inactivating more than three or four log units of faecal coliforms in waste-water effluents. Paradoxically, there was relatively little response in the unfiltered samples at UV dosages greater than 12 MW.s.cm^{-2} (Qualls *et al.* 1983). Since SS may scatter as well as absorb UV light, the average intensity in the reactor is usually higher than predicted by the photometric method.

UV transmittance through the effluent should be measured at the predominant wavelength emitted by the UV lamp (253.7 nm). Average UV transmittance are calculated using Lambert's - Beer's Law (Meulemans, 1987), a relationship that accounts for the reduction of UV light through the depth of a water sample as shown in the following equation:

$$\log_{10}\frac{I}{I_0} = -e.l.C \tag{2.8}$$

where $\log_{10}\frac{I}{I_0} = Absorbance = \frac{1}{Transmittance}$; I_0 = intensity of the incident light; I = intensity of the transmitted light; l = path length of absorbing solution; C = concentration of the solution (mol.L^{-1})

Germicidal UV light energy emitted by a lamp is attenuated as the distance from the source increases. Attenuation is caused by two mechanisms: absorption and dissipation of UV light. For a sphere the surface area over which it is projected increases and is shown by the following expression:

$$I = \frac{S}{4\pi R^2} \tag{2.9}$$

where I = light intensity at distance R (cm) (watts.cm^{-2}) and S = output of UV energy (watts).

The presence of colour, turbidity, and organic and inorganic compounds in waste-water reduces transmittance. Hence, with increasing degree of treatment, transmittance generally improves, and industrial effluents typically have a lower UV transmittance than domestic effluents. Nieuwstad *et al.* (1991) demonstrated that the relationship between the average UV intensity (E) and water transmittance at 254 nm (Tr) could be linearized by the following equation:

$$\ln E = pTr + q \tag{2.10}$$

where p and q = constants.

They also found that the correlation coefficient was higher than 0.993 in the region 10-90% transmittance.

In order to vary transmittance, sodium thio-sulfate or adenosine was used (Sommer & Cabaj, 1993), whereas instant coffee was used in the studies of Davey *et al.* (1995). In other studies by Whitby *et al.* (1993), the UV transmittance was changed by varying the flow rate of a reservoir of Para-HydroxyBenzoic (PHB) dissolved in de-ionised water.

Bacterial recovery following exposure to UV has been known for some 40 years. The extent of bacterial repair and re-growth after disinfection is expressed by calculating a repair rate (RR) following the relationship (Kelner, 1951; Cairns, 1993):

$$RR = \frac{N_{pr} - N}{N_0 - N} \tag{2.11}$$

where N_0= Effluent concentration (MNP/100 mL); N = Effluent concentration before repair (MNP/100mL; N_{pr} = Effluent concentration after repair (MNP/100 mL)

Figure. 2.7 a-b illustrates that, under both light and dark storage conditions, the numbers of cultivable *E. coli* (following a 4 minute UV disinfection) can increase from 2 to nearly 8 after 6 days storage (Mechsner *et al.* 1991). Similarly, Baron *et al.* (1996) found an 82%

increase in the number of *E. coli* observed after a 3-hour incubation post exposure. Furthermore, the repair rate of *E. coli* decreased when the initial UV irradiation dose was increased (Baron & Bourbigot, 1996).

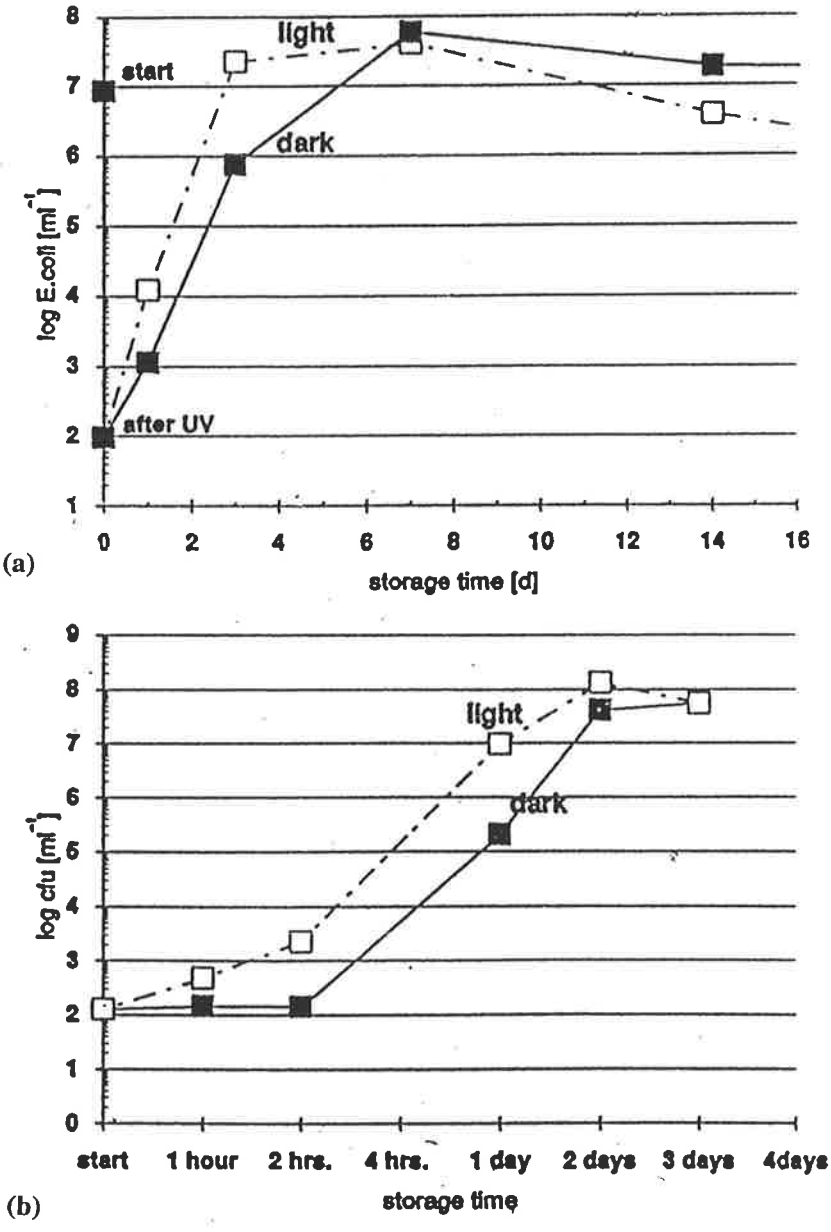
However, there is apparently no inverse relationship between the repair rate and UV dose (Scheible *et al.* 1986 and Harris *et al.* 1987b). For the same range of UV dose, greater increases were obtained for lower bacteria residuals, final densities thus remained low even after repair. Although the repair rate decreased when the % UV transmittance increased, no effect of SS on effluent bacterial concentration was found.

Similar responses were obtained for both a pure laboratory culture of *E. coli* and a natural stream sample, however, re-growth was clearly observed after the first few hours following treatment. Furthermore, it was evident that the survival of bacteria from waste-water suspended 1% in ground-water increased approximately by a factor of 4 within a short-time following UV irradiation (Mechsner *et al.* 1991). Based on the presented data, the researchers concluded that UV treated water includes inactivated separate bacteria (*E. coli*) or mixed bacterial populations should be re-evaluated. This is in agreement with Chrtek and Popp (1991) who proposed that assessment the degree of recovery of bacteria by photo-reactivation after UV irradiation is essential. In this research, the concentration of faecal indicator bacteria and plate counts were examined immediately after UV irradiation and after one, two, three and four hours. The results showed that re-growth caused an increase in the density of coliform bacteria of about 1 $\log_{10}$ unit and of standard the plate count of about 0.7. It was noted faecal streptococci did not show any considerable photo-reactivation.

By contrast, Hengesbach *et al.* (1993) were unable to show any significant bacterial re-growth in UV disinfected drinking water, even after several months of storage. Moreover, practical experience with drinking water from reservoirs in the Western part of Germany suggests that the bacterial re-growth potential is negligible if the DOC is less than 1 $mg.L^{-1}$. Similarly, an investigation in a natural environment showed that the photo-repair of micro-organisms may not be a major concern with UV disinfection (Whitby & Palmateer, 1993). Since repair of UV treated virus is UV disinfection of airborne viruses, it was observed that

Figure 2.7 **Regrowth of (a) *E. coli* and (b) a natural bacterial mixture in stream water after 4 minutes UV irradiation during storage under light and dark conditions**

(Reproduced from Mechsner *et al.*, 1991)



no infectious virus was recovered from the lungs of mice challenged with the UV-irradiated virus (Jakab & Knight, 1982).

In case of using UV irradiation in combination with hydrogen peroxide no bacterial regrowth was found, even the total microbial count decreased approximately 50 % after 3 days storage (Carnimeo *et al.* 1995).

2.6 UV unit design

Most UV units presently available are similar in basic construction. They consist essentially of cylindrical reaction chamber with the UV lamp mounted along the centre axis. The sizing of a reactor depends critically on the flow-rate, the level of suspended solids in final the effluent, UV transmittance of the effluent, and the coliform level permitted after disinfection. Desirable features essential for avoiding flow problems or inadequate dosage of the required wavelength include:

- an integrator to record the lamp usage
- an indicator for lamp failure
- an temperature sensor to monitor the temperature between the reactor sleeve and the lamp
- a water flow sensor and controller to prevent under-dosing
- a UV intensity monitor
- a stand-by unit to conduct water round a faulty unit while maintenance is being carried out
- pre-filter units to improve transmission or reduce the risk of encapsulation
- solenoid valves to enable the water flow to be interrupted should there be a power failure or if a suitable alarm signal is received from one of the monitoring devices.

For UV disinfection of drinking water, technical equipment must be operated with UV sensors in place that monitor or even regulate the flow rate. A chemical actinometry can be used to calibrate such sensors to ensure proper dosage of the disinfecting radiation for safe disinfection and avoid not only high energy costs, but unwanted by-product formation.

Added safety can be built-in by employing post-treatment filters to remove inactivated micro-organisms.

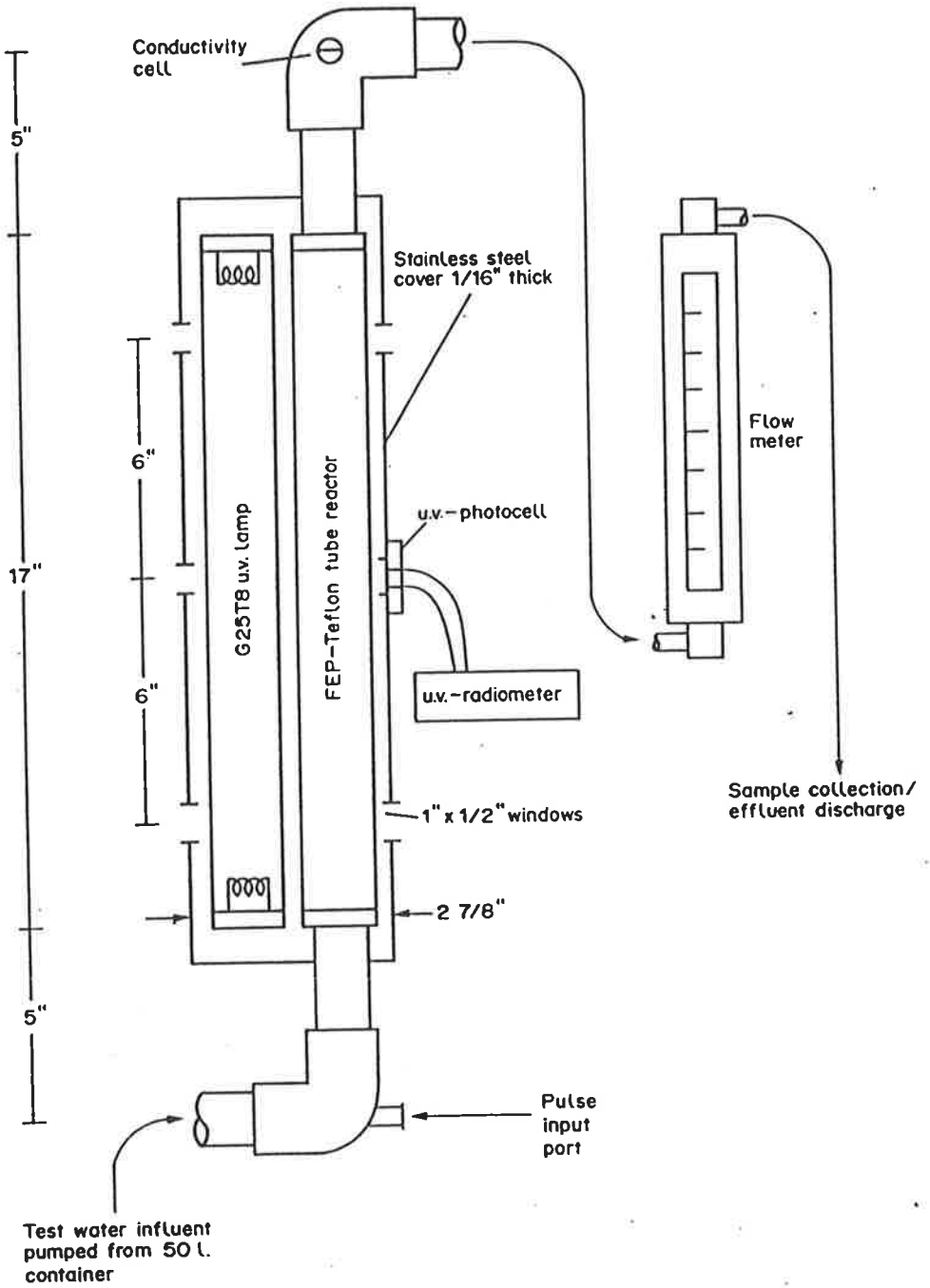
Two basic types of UV reactor designs prevail for municipal waste-water treatment systems. In one design, lamps enclosed in quartz glass sheaths are immersed in the waste-water flowing through a reactor. The second design involves conduction of waste-water through Teflon$^{(R)}$ or quartz tube externally irradiated by UV lamps (Thampi & Sorber, 1987). An example of a UV system using a Teflon tube reactor externally irradiated by a single UV lamp is shown in Figure 2.8.

Currently, low-pressure and medium pressure mercury vapour lamps are two types of commercial UV systems. Low-pressure lamps are the most efficient source of germicidal UV light because they produce a narrow band of UV light which peaks near the maximum germicidal wavelength of 260 nm. However, because of their greater UV output intensity, medium-pressure systems provide a much greater treatment capacity (approximately 25 times). The number of lamps required for disinfection depends on reactor design, water characteristics and permit requirements. However, closely spaced lamps are needed in UV inactivation of waste-water because of the high UV absorbance of waste-water (Qualls & Johnson, 1985).

Non-probabilistic and probabilistic methods are described to determine the number of lamps required for disinfection at a waste-water treatment plant (Loge *et al.* 1996).

Figure 2.8 **UV reactor experimental set-up**

(Adapted from Thampi & Sorber, 1985)



2.7 Economics of UV disinfection

The operating costs of UV disinfection can be divided into three categories:

- power
- lamp replacement
- labour costs.

If the work hours required to maintain a UV system was assumed not to be significant, annual operating cost for a UV system with capacity of 10,000 $m^3.day^{-1}$ was US $9,000 (Maarschalkerweerd *et al.* 1990). However, maintenance by trained personal is essential; particularly in component UV lamp assembly. Lamp cleaning costs vary with equipment types, but are not considered significant compared with power usage and lamp replacement costs.

It has been proposed that the cost of UV disinfection can be approximated from required contact time and the length of UV lamp per liquid reactor volume in the reactor design. Significant amounts of money could be saved if UV systems consisting of two or more tanks containing the UV lamps as well as devices for maintaining a constant water level, were used.

Thousands of dollars per year savings in power cost can be obtained by cycling tanks on or off line according to effluent/water flow rates. Where UV radiation was used in combination with hydrogen peroxide, the total cost was US $19.5 /1000$m^3$ (Carnimeo *et al.* 1995). For well-treated secondary effluents with flows between 0.044 and 4.4 $m^3.s^{-1}$, cost (capital plus operating) was estimated to range between US $12.00 and US $8.00/1000$m^3$, respectively (Venosa, 1983). It was expected that optimising H_2O_2 doses and points of addition could further reduce operating expenses.

The UV transmittance of water to be treated also has a considerable impact on the cost of UV disinfection. Whilst disinfection of a sand-filtered effluent, secondary effluent and good-quality effluent might be expected to cost approximately US $0.015, $0.0079, and US $0.0072 per 1000 US gallons, respectively, in case of a secondary effluent with 70%

transmittance costed US $0.021 per 1000 gallons based on lamp replacement and power costs (Severin, 1980). Thus acceptable UV transmittance not only affects disinfection efficiency but also savings in disinfection costs.

Based on previous investigations UV disinfection is believed to be cheaper than all commercially available disinfectants, except chlorine. Oliver *et al.* (1975) calculated total operational costs of ultraviolet systems and reported that in comparison with chlorination, ultraviolet treatment was more expensive (~1.2 US cents per 1000 US gallons, whereas the cost of the chlorine needed for disinfecting 1000 US gallons of secondary effluent from 0.1US cents for larger plants to about 0.5US cents for smaller facilities). Even so, UV treatment is used as an alternative to chlorination because of environmental advantages.

On the other hand, some other investigators have suggested UV disinfection could be economically competitive with chlorination. However, in cost comparisons between using UV or ozone alone to chlorine, ozone was rejected due to higher operational cost. Venosa *et al* (1984) on the other hand has reported that sequential application of ozone-UV or UV-ozone was more economical than either UV or ozone alone.

2.8 Summary and concluding remarks

It is clear that high level of suspended solids (SS) and water soluble materials which absorb UV light, limit application of this environmentally attractive disinfection system. Particulate and colloids limit transmittance and probably protect bacteria and viruses from UV light. UV absorbing materials also place limits on the effective dose of UV delivered. Sub-lethally injured bacteria which remain in treated water can repair UV induced damage and therefore contribute to a potential public health risk.

Notwithstanding these issues, critical analysis of the published literature shows that UV disinfection systems:

- are the next best alternative to chlorination and can be used to disinfect both potable water and secondary effluent

- in combination with oxidants represent a powerful disinfection system for removal of viable micro-organisms and non-biological organic matters from potable and waste-waters.

However, there is a distinct lack of quantitative information about:

- whether UV disinfection follows first or second-order kinetics
- mechanisms to measure the UV dosage reliably and consistently. Furthermore, short-circuiting through the UV chambers which causes the actual detention time to be greater than the theoretical detention time
- thc impact of water UV transmittance and SS even though these factors have been considered to be major limiting factors of UV disinfection. Little published information is available which correlates the effect of UV transmittance and suspended solids with efficacy of UV disinfection. In particular the effect of these factors on the kinetics and efficiency of UV disinfection have not been studied
- The acquisition of quantitative data on the effect of UV absorption and SS on the efficacy of UV disinfection could be justified by an increased confidence an application and understanding and as a necessary step to process optimisation.

CHAPTER 3 - MATERIALS AND METHODS

3.1 UV disinfection unit UV-LC5^{TM}

A commercial UV disinfection unit was selected for this study, a UVTA Model LC5^{TM} (Ultraviolet Technology of Australasia P/L., Glynde, South Australia), that was designed for a maximum flow rate of 4-5 L.min^{-1} for drinking water and 2 L.min^{-1} for industrial waste-water. The UV-LC5 unit includes one germicidal UV mercury vapour lamp (high pressure, 552 kpa) with a U - tube (made from an advanced fluoropolymer$^{(R)}$) which carries water along the side of the UV mercury vapour lamp. The lamp and the fluoropolymer tube are enclosed in robust, powder-coated aluminium case. The mercury vapour lamp is mounted in an easy-turn lamp holder to permit quick and easy replacement.

The UV-LC5 has a safety switch which turns the power off to the lamp prior to opening the unit. A clear glowing "jewel" on the outer enclosure is designed for visual observation of lamp operation. Details of the UV-LC5 unit are shown schematically in Figure 3.1. Some features and operating characteristics of the LC5 are given in Table 3.1.

3.2 Flow system

3.2.1 Experimental set up

The unit was connected to a flow-loop with feed-tank (20 litre glass) of Reverse Osmosis (RO) water, centrifugal pump (PV 52, James Beresford & Sons Ltd., Birmingham, England), variable-area flowmeters (Platon VA marked linearly from 0.4 to 4.4 L. min^{-1} , ABB Kent Taylor, England) and flow control valve (Swagelok$^{(TM)}$, Adelaide Valve & Fitting, South Australia). The experimental flow-loop is shown schematically in Figure 3.2. A photograph of the flow-loop is presented as Figure 3.3.

Table 3.1 Some features of Model UV-LC5 disinfection unit
(UVTA P/L., South Australia)

Flow rate (subject to quality)	1 to 5 L. min^{-1}
Maximum flow	5 litre per minute
Lamp type	20W low pressure mercury vapour
U advanced fluoropolymer tubing	11.11 mm inside diameter, 636.6 mm axial length
Inlet/outlet pine	12.7 mm outside diameter
Maximum operating pressure	552 kpa at @ 24 °C (less 2% for each 1°C rise)
Power requirement	240V 50 Hz 0.37 A
Unit dimensions	340 mm length x 100 mm wide x 75 mm height
Shipping weight	2 kilogram

Figure 3.1 **UV-LC5 disinfection unit**

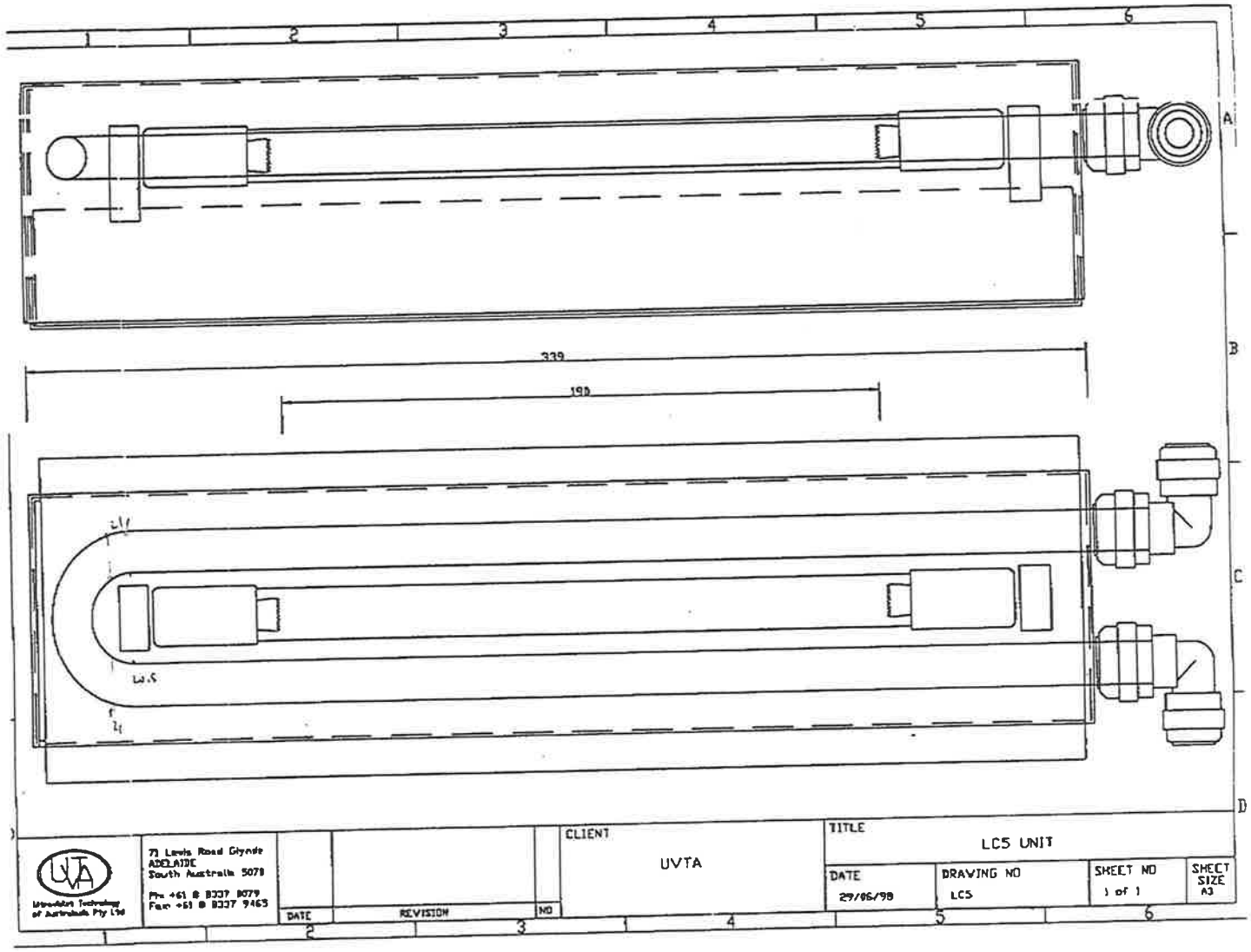


Figure 3.2 Flow diagram of the experimental UV disinfection unit

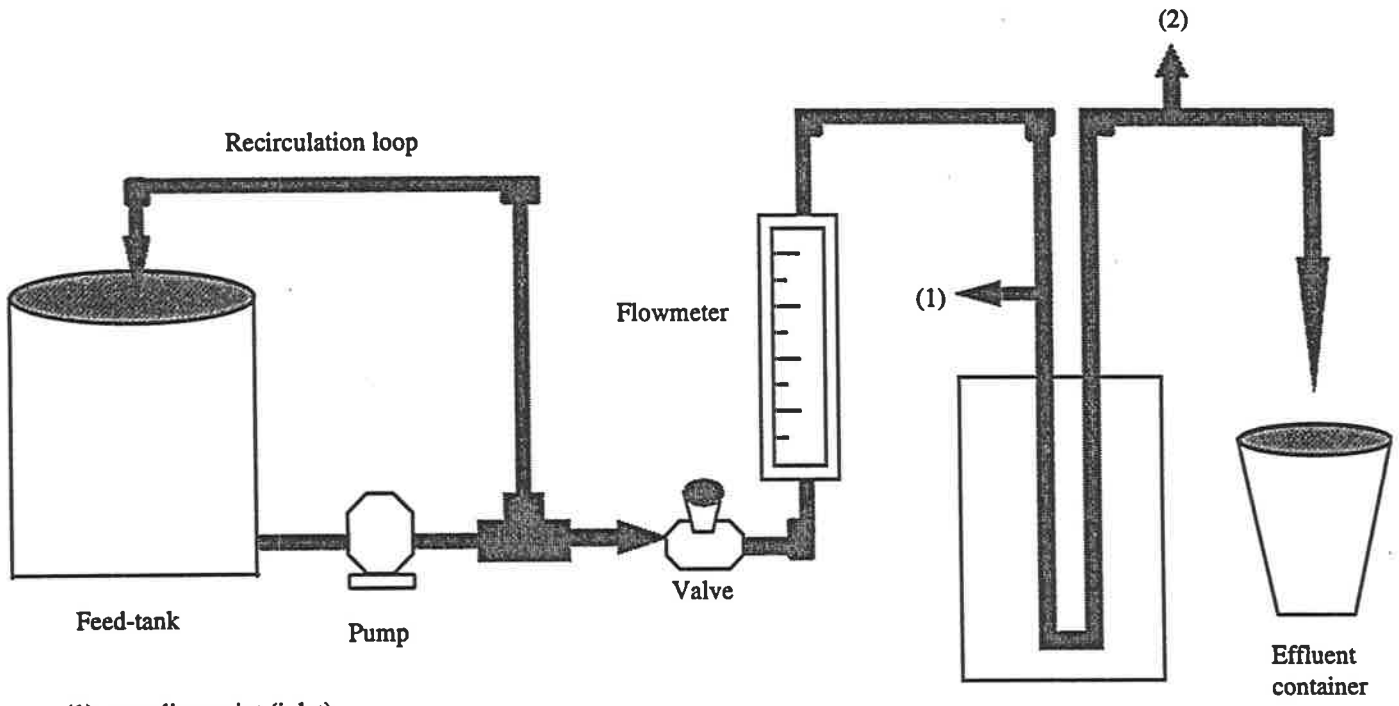
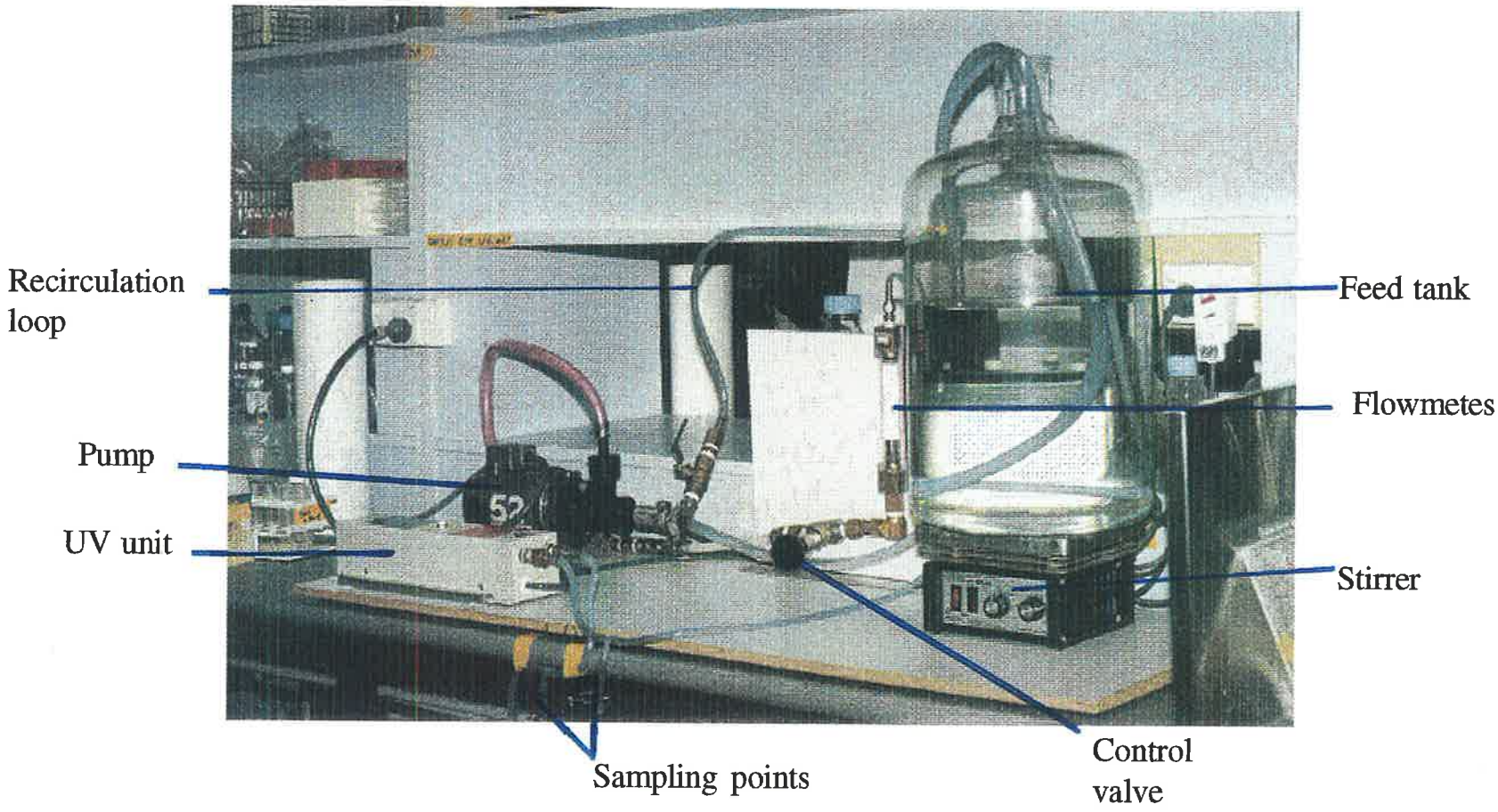


Figure 3.3 Photograph of the experimental flow-loop



3.2.2 *Selected bacterial contaminants*

Escherichia coli and *Pseudomonas aeruginosa* were chosen as the test micro-organisms. The particular strain of *E. coli* was the "FDA Seattle" strain, American Type Culture Collection (ATCC) number 25922. *E. coli* is a gram negative, motile rod, about 1mm by 2 to 3 mm in length (Stanier *et al.* 1972). *E. coli* was selected because it is found in sewage water contaminated with faecal material. It is used as an indicator for the presence of enteric pathogens and should not be present at all in potable water.

P. aeruginosa is a typical gram negative, aerobic bacterium, remarkably versatile in its ability to assimilate organic material and to perform biochemical activities. *P. aeruginosa* is considered to be an opportunistic pathogen. It is commonly found in moist environments and was selected as a test micro-organism because it has DNA comprising relatively high molar ratio of guanine (G) and cytosine (C) and therefore is more resistant to inactivation by UV light than is *E. coli* (Cano & Colome, 1986).

Cultures of both bacteria were obtained from the Department of Microbiology & Immunology, University of Adelaide collection. These test micro-organisms satisfied the essential requirements of:

- ready availability from accessible stocks
- significant sensitivity to ultraviolet irradiation
- simple growth requirements
- easy dispersion as individual cells.

3.2.3 *Absorbing agent and suspended solids*

In order to vary UV transmittance of the feed-tank water the addition of a UV absorbing material (International Roast™- Instant coffee power) or a suspended solid (Diatomaceous earth as Celite 503™, Ace Chemical Co., South Australia) were used.

The median particle size of Celite 503 is 23 μm. Celite 503 consists of 89% SiO_2 (silica).

Instant coffee is the dried water soluble extract of roasted, ground coffee, which readily dissolves in both cold and hot water.

The non-volatile and volatile components identified (using HPLC -High Pressure non-volatile Liquid Chromatography methods) in instant coffee include caffeine, chlorogenic and other acids (non-volatile); carbonyl compounds, alcohols, acids, esters, heterocyclic and aromatic compounds (volatile) (Trugo *et al.* 1983; Dart & Nursten, 1985). Ultraviolet irradiation may be absorbed by those compounds containing conjugated bonds. When added to water at equivalent concentrations, the UV absorbance of a coffee solution is greater than that of Celite (Appendix G presents ultraviolet and visible action spectra of coffee or Celite solution and in the presence of bacteria).

3.3 UV inactivation methods

The UV inactivation efficacy of *E. coli* and *P. aeruginosa* in RO water (with or without the presence of suspended solids or a UV absorbing agent) was evaluated using the experimental disinfection unit (Figures 3.1 to 3.3). The experimental study involved:

- the production and harvesting of the test micro-organisms
- UV inactivation of the test micro-organisms in the feed tank water using LC5™ unit
- the counting of viable cells by a Standard Plate Count method.

The details of these procedures are given below.

3.3.1 Production and harvesting of the test micro-organisms

Nutrient Broth (NB) consisted of 10 g Oxoid peptone, 10 g Oxoid Lab Lemco powder and 5 gram NaCl dissolved in distilled water to make 1 litre. The neck of the flask was plugged with cotton wool bung, then autoclaved at 121°C for 30 min. Nutrient agar (NA) consisted of Nutrient Broth solidified with addition of 15 $g.L^{-1}$ Oxoid agar.

The test bacteria were routinely prepared from glycerol stock cultures maintained at -70°C. A loop full of frozen culture was plated on the surface of NA and the plate incubated overnight at 37°C. A colony was selected and used to inoculate 50 mL of NB. After overnight incubation at 37°C, the whole culture was used as an inoculum for a 2 L volume of NB. The flask was incubated with shaking at 37°C for about 7 h.

Bacterial cells were then harvested by centrifugation (Beckman JA 10 rotor at 7000 rpm and 4°C for 10 min). The cell pellet was resuspended in saline solution, then added into the feed tank water to give initial viable numbers (N_0) of between 10^6 to 10^8 cells per mL.

3.3.2 UV inactivation of test micro-organisms

The feed-tank water with a test micro-organism added was pumped through the disinfection unit. For each flow-rate, four sample tubes were taken before and after UV exposure and placed in an ice bath (at ~ 4°C) in sealed containers for subsequent plate counts to enumerate viable cells. The flow-rate was monitored using variable-area flow-meters. During each trial, the flow-rate was adjusted using control valve to values of 1, 2, 3 or 4 L.min^{-1}. The flow-meter was calibrated by recording the volumes and mass of water collected over time for each of the flow settings against a stopwatch.

Water in the feed-tank was pumped through a re-circulation loop, and a stirrer was applied to mix the tank contents and obtain an even distribution of micro-organisms in the feed-tank.

3.3.3 Enumeration of viable bacteria using Standard Plate Count Method

The plate count, or colony count, is a standard procedure for enumerating viable micro-organism populations. In this method, it is assumed that each viable cell will yield one colony. Samples of *E. coli* and *P. aeruginosa* -test liquid suspension exposed to UV light, and control preparations, were diluted with saline solution. Serial ten-fold dilutions were made with 0.1 mL transfers into 0.9 mL solution. This constant dilution factor was used throughout the experiments to reduce the chance of error when making a large number of counts. Sterile 1.5 mL plastic reaction tubes were used for preparation of dilutions. The reaction tubes were thoroughly mixed using a vortex mixer on each successive dilution. Volumes (0.1 mL) of an appropriately diluted culture were then spread over the surface of pre-dried agar plate using a sterile glass spreader. The plates were then incubated for 20 - 24 h at 37 °C. Plates with between 30 and 300 colonies were counted. The count of viable bacteria per mL of liquid suspension was calculated as the mean number of colonies per dilution plate multiplied by the reciprocal of the dilution factor.

3.3.4 Determination of UV dose

Ultraviolet dose was determined as the product of intensity and residence time (*see* equation 2.7) and using bioassay method as follows:

3.3.4.1 UV intensity

UV intensity was calculated by a two dimensional mathematical model (*see* Appendix C). The model UV-LC5 disinfection unit provides an intensity of 11,940 $\mu W.cm^{-2}$ (UVTA, *pers. comm.*). This value was used to estimate UV dose in all subsequent analyses.

3.3.4.2 Bioassay method

The basic procedural steps for the biological assay were carried out similar to that used by Braunstein *et al* (1996). Bioassay tests compare survival rates of a specific microbial agent in a static UV test and in tests where the micro-organisms are passed through a UV reactor at given flow rate. Although the bioassay method probably require refinement, particularly in regard to standardization of the methods and procedures, bioassay tests provide a reasonably reliable method for verification of dose supplied by various UV reactors (Crandall, 1986).

E. coli was selected for the basis of the bioassay of UV dose. Test suspensions were exposed to UV light during passage through the UV-LC5 disinfection unit. The survival of the test bacterium was determined as a function of UV dose in order to "standardise" the sensitivity of the bacteria. The initial concentration of *E. coli* in the sample was approximately 10^7 cells per mL.

Samples of irradiated suspension were then either serially diluted (as described above) or plated directly onto the surface of NA. All plates were then incubated at 37 °C for approximately 20 h. our replicate plates were made for each assay. After incubation the number of cells in each plate were counted. The counts on four replicate plates were averaged and recorded as number of cells per mL of bacterial suspension.

The UV dose was varied by changing the flow rate. The residence time was calculated by dividing the net reactor volume by the flow rate of water through the system. The net reactor volume is equal to the volume of water contained over the U tube axial length (*see* Appendix D).

3.3.5 UV transmittance measurement

UV transmittance of feed tank water was measured in a 1 cm cuvette using a Beckman DU-65 Spectrophotometer (Beckman Instruments Inc. Fullerton, CA 92634). All measurements - single wavelength were made at the 253.7 nm setting or scanned at 200 - 400 nm, and UV transmittance (%) values were read at range of 0-100%.

UV transmittance is the inverse of UV absorption. Absorption of UV light is related to the absorptive properties of the medium through which it is transmitted. Further, transmittance is reduced by colour, suspended solids, and organic and inorganic compounds present in the waste-water (Mann, 1992).

3.3.6 Bacterial repair following UV exposure

The re-growth experiments were carried out with the two test micro-organisms: *E. coli* and *P. aeruginosa*. The UV disinfected water was collected in sterilised 50 mL containers, and stored for up to 6 days under the following different conditions:

- at room temperature (~ 22°C)
- at 30°C darkness or light
- at 37°C darkness or light.

Samples were withdrawn after 1 h, and 1, 3 and 6 days of storage and the number of viable bacteria determined using the Standard Plate Count Method. The mean values of four successive trials were plotted *vs.* the residence time and storage time (*see* Chapter 4).

3.3.7 pH measurement

The pH of the feed-tank water with the suspended cells was taken before and after UV exposure. Measurements were conducted with standardised pH electrodes using TPS Digital pH Meter (TPS P/L., Brisbane, Australia). Calibration was carried out using standard, buffer solutions at pH 4 and 7.

3.4 A typical experiment

For each trial, typical experimental procedures involved the following steps:

- the 20L feed tank was sterilised (autoclaved at 121°C and 30 min) then aseptically filled with RO (Reverse osmosis) water. Two control (10 mL) samples of the feed-tank water were tested (using Standard Plate-Count Method - SPCM - microbiological practice) to ensure that no viable cells contaminated the flow-loop
- a specified volume and number of test micro-organisms was then added from a glass container to the feed-tank to provide a total number of micro-organisms in the feed tank water between 10^6 to 10^8 cells per mL
- UV absorbing agent (International Roast™- Instant Coffee-powder, 0.001-0.03 $g.L^{-1}$) or a suspended solid (Diatomaceous earth - as Celite 503™, 0.01 - 0.3 $g.L^{-1}$) was then added to the feed-tank water to vary UV transmittance between 30 and 80%. To avoid any potential for microbial contamination in the experimental loop, appropriate solutions of coffee-powder and Celite 503 were heated to 70 °C for 10 min, then cooled to room temperature prior to addition to the feed-tank
- water in the feed-tank was pumped through a recirculation loop to mix the tank contents and obtain an even distribution of micro-organism cells in the feed-tank water. A magnetic stirrer plate was used to ensure an even distribution of Celite 503 within the test suspension
- two samples of the water in the feed tank were taken for measurement of pH and UV transmittance
- water was then pumped through the UV disinfection unit.
- the flow rate was adjusted to 1.0, 2.0, 3.0, or 4.0 $L.min^{-1}$ to give, respectively, residence times of 3.7, 1.9, 1.2 and 0.9 s
- at each flow rate, replicate samples of water both entering and leaving the UV disinfection unit were taken from sampling points
- all water samples taken were stored in an ice bath (~ 4°C) for subsequent analysis
- initial an surviving numbers of viable test micro-organism were enumerated using SPCM.

CHAPTER 4 - RESULTS AND DISCUSSION

4.1 UV inactivation of test micro-organisms

4.1.1 Review of the major experimental data

A summary set of typical detailed experimental results for survival of both *E. coli* and *P. aeruginosa*, each with four UV doses and four concentrations of UV adsorbing material (coffee) or suspended solids (Celite 503) are presented in Tables 4.1a to 4.1d. There are 72 separate experimental conditions. Each separate experimental condition was replicated 3 or 4 times giving a total of 260 individual experiments (*see* Appendix E).

The UV dose as a function of flow-rate and residence (exposure) time of the feed-tank water within the UV-LC5 disinfection unit are summarised in Table 4.2. The UV dose ranged from 10,800 to 44,200 $\mu Ws.cm^{-2}$.

During experiments with the UV-LC5 disinfection unit the pH of the feed-water varied between 5.1 and 6.7 - depending on the concentration of coffee or Celite 503 added. This range of values of pH is not considered to have any significant impact on the viability of the suspensions of the two test micro-organisms. Further, the temperature of feed-tank water ranged from 20 to 24 °C. It is known that this factor has very little effect on UV inactivation of contaminating bacteria in this range (Meulemans, 1987) and a control of feed-tank water temperature was not required.

Careful experiment established that neither coffee or Celite at the concentrations used had a significant biocidal effect on either type of micro-organism used to inoculate feed-tank water. The initial number of viable cells in the feed-water tank was not effected after the addition of the UV adsorbing agent and SS over a period of some 3 h. Reductions in the number of viable bacterial cells therefore are all attributable to UV exposure (*see* Appendix I).

Further, it was important to establish whether Celite adsorbed significant numbers of bacterial cells. Adsorption of significant numbers of viable bacterial cells could skew the survival data.

Sorption of bacteria to Celite particles, could have two effects:

- firstly, the adsorbed bacteria could be protected from UV radiation.
- secondly those cells adsorbed would be underestimated in Standard Plate Counts - and thus give rise to an apparently greater disinfection efficacy than is actually the case.

To test the possibility of adsorption effects, a suspension of cells of *E. coli* was mixed with Celite of some ten-times greater concentration than used in the disinfection studies (~ 3 gL^{-1} *cf* 0.3 gL^{-1}). A portion of the suspension of viable *E. coli* cells was used as a control. Following centrifugation and a four-fold wash to separate Celite and bacterial cells the number of viable cells in the supernatant were enumerated - and also in the control sample. This test experiment was replicated four times with each of *E. coli* and *P. aeruginosa* suspensions. These experimental test data are given in Appendix I.

As there was no significant difference between the numbers of viable cells recovered from the supernatant and that from a control experiment, it was concluded that Celite did not adsorb significant numbers of viable cells of either test micro-organism. Thus reductions in viable cell numbers could be attributed wholly to UV irradiation in the disinfection unit.

The overall efficacy of a UV disinfection unit can be determined from a simple plot of numbers of survivors *vs* the residence time. Plots of percent survival (as $100 \times N/N_0$) *vs.* residence time (t) from Tables 4.1a to 4.1d are presented in Figures 4.1a to 4.1b at each of five values of feed-water transmittance. At the four flow settings (1, 2, 3, and 4 $L.min^{-1}$), the residence time of the contaminated water was respectively, 3.7, 1.9, 1.2 and 0.9 s. As expected, these figures show that the % survival for both *E. coli* and *P. aeruginosa* increased with decreasing residence time and decreasing UV transmittance of the feed-water. For example, for *P. aeruginosa* in the presence of Celite at a mid-range residence time of 1.9 s, a five-fold increase in survivors was obtained by reducing UV transmittance of the feed-water from 79% to 57%. In contrast, an approximately 9 fold reduction of *E. coli* was obtained under identical range of feed-water UV transmittance. This underscores that suspensions of *P. aeruginosa* are more resistant to UV radiation than are *E. coli*.

The curvature of Figure 4.1 is important as it implies an exponential relationship, or first-order kinetic model for disinfection inactivation kinetics.

Figure 4.2 illustrates the survival kinetics obtained for suspensions of both test strains of bacteria. The data are plotted as ln (N/N_0) vs UV dose (residence time) (*see* equation 2.6) at a mid-range value of UV transmittance of the feed-water of 65%. The UV transmittance (254 nm) of the feed-water used to suspend the bacteria was adjusted to 65% by addition of known amounts of coffee or Celite. Survival data are the mean value of the four replicates. Viewed this way, the greater the absolute number of $\ln N/N_0$ the smaller the number of survivors.

The figure shows a similar trend in survival of suspensions of both test bacteria in the presence of either coffee (UV absorbing) or Celite 503 (suspended solids). This figure shows also that survival of suspensions of both test bacteria decreased with increased UV dose. It was expected that the survival of *P. aeruginosa* would be greater than that for *E. coli* . This is shown to be the case and is seen most clearly at the higher UV doses. At residence times less than the practical minimum obtainable with the UV-LC5 disinfection unit (<0.9 s), the curve has been artificially extended to the zero-time intercept. The portion of the curve covering the experimentally data appears highly linear for combinations of both test bacteria and UV absorbing agent and suspended solids. This implies first-order kinetics of UV inactivation over the experimental range of UV dose (residence time).

The dependence of inactivation efficacy on residence time and feed-water UV transmittance over the range 52 - 70%, is highlighted in Figure 4.3 for both bacterial types at, respectively, 99.99% and 99.97% inactivation (0.01% and 0.03% survival). At a UV transmittance of 52 %, an increase in residence time from 1.9 to 4.5 s is necessary for an increase in inactivation of *E. coli* from 99.97 to 99.99 %. Similarly, an increase in residence time from 3.7 to 5.6 s is necessary for *P. aeruginosa*. As the feed-water transmittance is increased, the effective UV dose (or residence time) needed to ensure a specified level of inactivation is reduced. At a value of transmittance of 70%, the time necessary to disinfect viable numbers of contaminating cells of *E. coli* by 99.99 % is about 1.5 s; about 1.7 times this residence time is required for a similar reduction in numbers of *P. aeruginosa* (Figure 4.3). Furthermore, the increased residence time necessary for

inactivation of viable cells of *P. aeruginosa* is constant over the entire range of values of UV transmittance tested.

Experimental survival data obtained for *E. coli* with the UV-LC5 disinfection unit are in general agreement with those of other published data, for example, Savolainen (1991). However Crandall (1986), reported significantly lower UV doses for a given inactivation of both *E. coli* and *P. aeruginosa*. A 100% inactivation of both *E. coli* and *P. aeruginosa* required a UV dose of 6,600 $\mu Ws.cm^{-2}$ and 10,500 $\mu Ws.cm^{-2}$, respectively. his compares with values obtained with the UV-LC5 disinfection unit of, respectively, about 24,500 $\mu Ws.cm^{-2}$ and 44,200 $\mu Ws.cm^{-2}$ for a 99.99% inactivation at a near mid-range transmittance of 66%. A difficulty with such direct comparisons with the literature includes the fact that most reported data are fragmentary in that the value of transmittance, pH and other factors are not given.

Survival data from Figure 4.3 together with additional data are tabulated in Table 4.3 to give a convenient summary of the residence time and UV transmittance necessary to result in a given inactivation of suspensions of both test bacteria over a range from 99.95% to 99.99% survival. At the mid-range residence time of 1.9 s and lowest UV transmittance of feed tank water (52%), the inactivation efficacy of *E. coli* and *P. aeruginosa* was 99.98% and 99.95%, respectively.

Table 4.1 (a) UV inactivation and microbiological results

UV dose = 10,800 μW.s.cm^{-2}, t = 0.9 s, flow = 4 L.min^{-1}

Disinfection trial	1	2	3	4	5	6
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
Coffee or Celite added (g.L^{-1})	0 coffee	0.001 coffee	0.005 coffee	0.01 coffee	0.03 coffee	0.01 Celite
UV transmittance (%)	78	69	65	60	52	70
pH	5.95	5.76	5.85	5.44	5.62	6.13
Initial number, N_0 (No.mL^{-1})	88 x 10^6	16 x 10^7	22 x 10^7	20 x 10^6	40 x 10^6	79 x 10^6
Survival number, N (No.mL^{-1})	30 x 10^2	10 x 10^3	23 x 10^3	33 x 10^2	122 x 10^2	132 x 10^2
% Survival (100 x N/N_0)	0.003	0.0065	0.0103	0.0165	0.0304	0.017
ln(N/N_0)	-10.29	-9.64	-9.18	-8.71	-8.1	-8.69
$\log_{10}(N/N_0)$	-4.47	-4.19	-3.99	-3.78	-3.52	-3.77

Disinfection trials	7	8	9	10	11	12
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added (g.L^{-1})	0.05 Celite	0.1 Celite	0.3 Celite	0 coffee	0.001 coffee	0.005 coffee
UV transmittance (%)	66	61	55	80	71	66
pH	6.06	6.03	5.90	5.38	5.51	5.93
Initial number, N_0 (No.mL^{-1})	31 x 10^7	80 x 10^7	24 x 10^7	98 x 10^6	90 x 10^6	80 x 10^6
Survival number, N (No.mL^{-1})	64 x 10^3	280 x 10^3	116 x 10^3	130 x 10^2	260 x 10^2	270 x 10^2
% Survival (100 x N/N_0)	0.0207	0.0352	0.0482	0.0126	0.0285	0.0336
ln(N/N_0)	-8.48	-7.95	-7.64	-8.98	-8.16	-7.99
$\log_{10}(N/N_0)$	-3.68	-3.45	-3.32	-3.90	-3.54	-3.47

Disinfection trials	13	14	15	16	17	18
Test micro-organism	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added (g.L^{-1})	0.01 coffee	0.03 coffee	0.01 Celite	0.05 Celite	0.1 Celite	0.3 Celite
UV transmittance (%)	62	55	72	65	61	53
pH	5.58	5.98	6.23	6.26	6.17	6.02
Initial number, N_0 (No.mL^{-1})	36 x 10^6	38 x 10^6	900 x 10^6	70 x 10^6	106 x 10^6	34 x 10^6
Survival number, N (No.mL^{-1})	150 x 10^2	210 x 10^2	34 x 10^2	300 x 10^2	53 x 10^2	250 x 10^2
% Survival (100 x N/N_0)	0.0416	0.0564	0.038	0.0429	0.0503	0.0721
ln(N/N_0)	-7.78	-7.48	-7.88	-7.75	-7.59	-7.24
$\log_{10}(N/N_0)$	-3.38	-3.25	-3.42	-3.36	-3.30	-3.14

Table 4.1 (b) UV inactivation and microbiological results.

UV dose = 14,100 μW.s.cm^{-2}, t = 1.2 s, flow = 3 L.min^{-1}

Disinfection trial	19	20	21	22	23	24
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
Coffee or Celite added (g.L^{-1})	0 coffee	0.001 coffee	0.005 coffee	0.01 coffee	0.03 coffee	0.01 Celite
UV transmittance (%)	78	69	65	60	52	70
pH	5.95	5.76	5.85	5.44	5.62	6.13
Initial number, N_0 (No.mL^{-1})	64 x 10^6	170 x 10^6	130 x 10^6	50 x 10^6	40 x 10^6	90 x 10^6
Survival number, N (No.mL^{-1})	20 x 10^2	10 x 10^2	124 x 10^2	69 x 10^2	100 x 10^2	122 x 10^2
% Survival (100 x N/N_0)	0.0029	0.0057	0.0095	0.0138	0.025	0.0136
ln(N/N_0)	-10.45	-9.77	-9.26	-8.89	-8.29	-8.90
log$_{10}$(N/N_0)	-4.54	-4.24	-4.02	-3.86	-3.60	-3.86

Disinfection trial	25	26	27	28	29	30
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added (g.L^{-1})	0.05 Celite	0.1 Celite	0.3 Celite	0 coffee	0.001 coffee	0.005 coffee
UV transmittance (%)	66	61	55	80	71	66
pH	6.06	6.03	5.90	5.38	5.51	5.93
Initial number, N_0 (No.mL^{-1})	120 x 10^7	160 x 10^7	31 x 10^7	112 x 10^5	70 x 10^6	50 x 10^6
Survival number, N (No.mL^{-1})	150 x 10^3	42 x 10^4	113 x 10^3	15 x 10^2	170 x 10^2	140 x 10^2
% Survival (100 x N/N_0)	0.0126	0.0263	0.0365	0.0130	0.0225	0.0279
ln(N/N_0)	-8.98	-8.24	-7.92	-8.95	-8.40	-8.18
log$_{10}$(N/N_0)	-3.90	-3.58	-3.44	-3.89	-3.65	-3.55

Disinfection trial	31	32	33	34	35	36
Test micro-organism	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added (g.L^{-1})	0.01 coffee	0.03 coffee	0.01 Celite	0.05 Celite	0.1 Celite	0.3 Celite
UV transmittance (%)	62	55	72	65	61	53
pH	5.58	5.98	6.23	6.26	6.17	6.02
Initial number, N_0 (No.mL^{-1})	35 x 10^6	54 x 10^6	70 x 10^5	90 x 10^6	86 x 10^6	44 x 10^6
Survival number, N (No.mL^{-1})	120 x 10^2	270 x 10^2	15 x 10^2	280 x 10^2	33 x 10^3	270 x 10^2
% Survival (100 x N/N_0)	0.0341	0.0504	0.0214	0.031	0.038	0.0601
ln(N/N_0)	-7.98	-7.59	-8.45	-8.08	-7.88	-7.42
log$_{10}$(N/N_0)	-3.46	-3.30	-3.67	-3.51	-3.42	-3.22

Table 4.1 (c) UV inactivation and microbiological results.

UV dose = 22,700 $\mu W.s.cm^{-2}$, t = 1.9 s, flow = 2 $L.min^{-1}$

Disinfection trials	37	38	39	40	41	42
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
Coffee or Celite added ($g.L^{-1}$)	0 coffee	0.001 coffee	0.005 coffee	0.01 coffee	0.03 coffee	0.01 Celite
UV transmittance (%)	78	69	65	60	52	70
pH	5.95	5.76	5.85	5.44	5.62	6.13
Initial number, N_0 ($No.mL^{-1}$)	46×10^6	180×10^6	130×10^5	50×10^6	30×10^6	100×10^6
Survival number, N ($No.mL^{-1}$)	20×10^2	100×10^2	87×10^2	48×10^2	61×10^3	74×10^2
% Survival ($100 \times N/N_0$)	0.0021	0.0056	0.0067	0.0096	0.0203	0.0074
$\ln(N/N_0)$	-10.77	-9.79	-9.61	-9.25	-8.50	-9.51
$\log_{10}(N/N_0)$	-4.68	-4.25	-4.17	-4.02	-3.69	-4.13

Disinfection trials	43	44	45	46	47	48
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added ($g.L^{-1}$)	0.05 Celite	0.1 Celite	0.3 Celite	0 coffee	0.001 coffee	0.005 coffee
UV transmittance (%)	66	61	55	80	71	66
pH	6.06	6.03	5.90	5.38	5.51	5.93
Initial number, N_0 ($No.mL^{-1}$)	55×10^7	700×10^7	50×10^7	81×10^6	80×10^6	90×10^6
Survival number, N ($No.mL^{-1}$)	56×10^3	96×10^3	162×10^3	69×10^2	11×10^3	160×10^2
% Survival ($100 \times N/N_0$)	0.0101	0.0137	0.0284	0.0085	0.0135	0.0175
$\ln(N/N_0)$	-9.20	-8.89	-8.17	-9.37	-8.91	-8.65
$\log_{10}(N/N_0)$	-3.99	-3.86	-3.55	-4.07	-3.87	-3.76

Disinfection trials	49	50	51	52	53	54
Test micro-organism	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added ($g.L^{-1}$)	0.01 coffee	0.03 coffee	0.01 Celite	0.05 Celite	0.1 Celite	0.3 Celite
UV transmittance (%)	62	55	72	65	61	53
pH	5.58	5.98	6.23	6.26	6.17	6.02
Initial number, N_0 ($No.mL^{-1}$)	80×10^6	45×10^6	110×10^5	104×10^6	80×10^6	56×10^6
Survival number, N ($No.mL^{-1}$)	240×10^2	150×10^2	154×10^2	200×10^2	23×10^3	260×10^2
% Survival ($100 \times N/N_0$)	0.0295	0.0341	0.014	0.0186	0.029	0.0458
$\ln(N/N_0)$	-8.13	-7.98	-8.87	-8.59	-8.15	-7.69
$\log_{10}(N/N_0)$	-3.53	-3.46	-3.85	-3.73	-3.54	-3.34

Table 4.1 (d) UV inactivation and microbiological results

UV dose = 44,200 μW.s.cm^{-2}, t = 3.7 s, flow = 1 L.min^{-1}

Disinfection trials	55	56	57	58	59	60
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*E. coli*
Coffee or Celite added (g.L^{-1})	0 coffee	0.001 coffee	0.005 coffee	0.01 coffee	0.03 coffee	0.01 Celite
UV transmittance (%)	78	69	65	60	52	70
pH	5.95	5.76	5.85	5.44	5.62	6.13
Initial number, N_0 (No.mL^{-1})	64 x 10^6	140 x 10^6	250 x 10^5	30 x 10^6	20 x 10^6	110 x 10^6
Survival number, N (No.mL^{-1})	7 x 10^2	37 x 10^2	88 x 10^2	15 x 10^2	20 x 10^3	30 x 10^2
% Survival (100 x N/N_0)	0.0011	0.0026	0.0035	0.0050	0.0100	0.0027
ln(N/N_0)	-11.42	-10.56	-10.26	-9.9	-9.21	-10.52
log$_{10}$(N/N_0)	-4.96	-4.58	-4.45	-4.30	-4.00	-4.57

Disinfection trials	61	62	63	64	65	66
Test micro-organism	*E. coli*	*E. coli*	*E. coli*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added (g.L^{-1})	0.05 Celite	0.1 Celite	0.3 Celite	0 coffee	0.001 coffee	0.005 coffee
UV transmittance (%)	66	61	55	80	71	66
pH	6.06	6.03	5.90	5.38	5.51	5.93
Initial number, N_0 (No.mL^{-1})	130 x 10^7	90 x 10^7	48 x 10^7	63 x 10^6	90 x 10^6	30 x 10^6
Survival number, N (No.mL^{-1})	66 x 10^3	80 x 10^3	82 x 10^3	10 x 10^2	40 x 10^2	19 x 10^2
% Survival (100 x N/N_0)	0.0051	0.0089	0.0171	0.0016	0.0044	0.0064
ln(N/N_0)	-9.88	-9.33	-8.67	-11.04	-10.03	-9.66
log$_{10}$(N/N_0)	-4.29	-4.05	-3.76	-4.79	-4.35	-4.19

Disinfection trials	67	68	69	70	71	72
Test micro-organism	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Coffee or Celite added (g.L^{-1})	0.01 coffee	0.03 coffee	0.01 Celite	0.05 Celite	0.1 Celite	0.3 Celite
UV transmittance (%)	62	55	72	65	61	53
pH	5.58	5.98	6.23	6.26	6.17	6.02
Initial number, N_0 (No.mL^{-1})	39 x 10^6	61 x 10^6	90 x 10^5	104 x 10^6	124 x 10^6	58 x 10^6
Survival number, N (No.mL^{-1})	37 x 10^2	73 x 10^2	67 x 10^2	94 x 10^2	24 x 10^3	160 x 10^2
% Survival (100 x N/N_0)	0.0095	0.0120	0.0074	0.0090	0.0189	0.0275
ln(N/N_0)	-9.26	-9.03	-9.51	-9.32	-8.57	-8.20
log$_{10}$(N/N_0)	-4.02	-3.92	-4.13	-4.05	-3.72	-3.56

4.1.2 Influence of initial numbers of contaminants

The initial densities of bacterial cells in the feed-tank water ranged from 10^6- to 10^8 cells.mL^{-1} (Table 4.1). This variation in initial cell density (N_0) might play an important role in the efficacy of UV inactivation.

To test this experimentally, a number of additional experiments were conducted with suspensions of both test bacteria at a fixed UV dosage of 10,800 μWs.cm^{-2}. Results from these inactivation trials are presented in Table 4.4. Although the absolute value of the number of inactivated cells appears to vary with initial bacterial viable cell numbers, importantly, the % survival - and the dimensionless inactivation ratio $\ln(N/N_0)$ - is not influenced by initial bacterial cell numbers (N_0).

Enhanced mixing in the disinfection unit should lead to a more intimate contact of a greater proportion of water containing the bacteria cells with the UV light. The Reynolds number for the corresponding flow rates of 1 - 4 L.min^{-1} in the UV-LC5 disinfection unit were calculated (*see* Appendix F). These calculations showed that over this flow range used in the UV disinfection unit, changes in the value of turbulence did not have a significant effect and hence was not considered further.

4.1.3 Comparison of the two test strains of bacteria

Comparison of the inactivation data for bacterial suspensions indicated extensive bacterial strain differences existed in the sensitivity to UV irradiation. As discussed in the previous section, it is evident that greater UV doses are required to inactivate *P. aeruginosa* than *E. coli*. This can be related to different UV sensitivities at 254 nm. The UV dose required for the reduction of *P. aeruginosa* by 99.99% was about 1.5 times greater than that required for *E. coli* (Figure 4.3). Furthermore, the survival of *E. coli* decreased only slightly with increasing UV doses in comparison to *P. aeruginosa*. In the presence of 0.3 g.L^{-1} Celite 503, the survival of *E. coli* varied between 0.038 % and 0.017 % as residence time was increased from 0.9 to 3.7 s (UV dose of 10,800 to 44,200 μWs.cm^{-2}, respectively), whereas the % survival of *P. aeruginosa* suspensions was reduced from 0.074% to 0.26% for the same change in residence time (Figure 4.1).

Clearly, the residual number of *E. coli* was not significantly dependant on UV dose or residence time. This may be due to small variations in the residence time intrinsic to the experimental apparatus used in this study. Differences between microbiological characteristics of *E. coli* and *P. aeruginosa* may also have contributed to this observation.

Remarkable differences in sensitivity to UV irradiation also existed between *E. coli* and *P. aeruginosa.* At the same UV dose and UV transmittance of the feed-tank water, *P. aeruginosa* demonstrated higher resistance to UV irradiation. This phenomenon is in part undoubtedly due to the fact that *P. aeruginosa* has higher percentage of guanine and cytosine (67 mole % G+C) in comparison with *E. coli* (44 mole % G+C). Thus, the proportion of UV hits forming potentially lethal thymine dimers on the same DNA strand will be less for *P. aeruginosa* and consequently this organism will be more resistant to UV radiation.

However, in our experiments, both *E. coli* and *P. aeruginosa* showed similar responses to changes in the concentration of UV absorbing agent (coffee) or suspended solids (Celite 503). At a flow rate of 2 $L.min^{-1}$ (UV dose of 22,700 $\mu Ws.cm^{-2}$) and concentrations of Celite 503 of 0.3 to 0.01 $g.L^{-1}$, the % survival of *E. coli* and *P. aeruginosa* were reduced by approximately a factor of 3 times (from 0.0024 to 0.00075 and from 0.0044 to 0.0015, respectively).

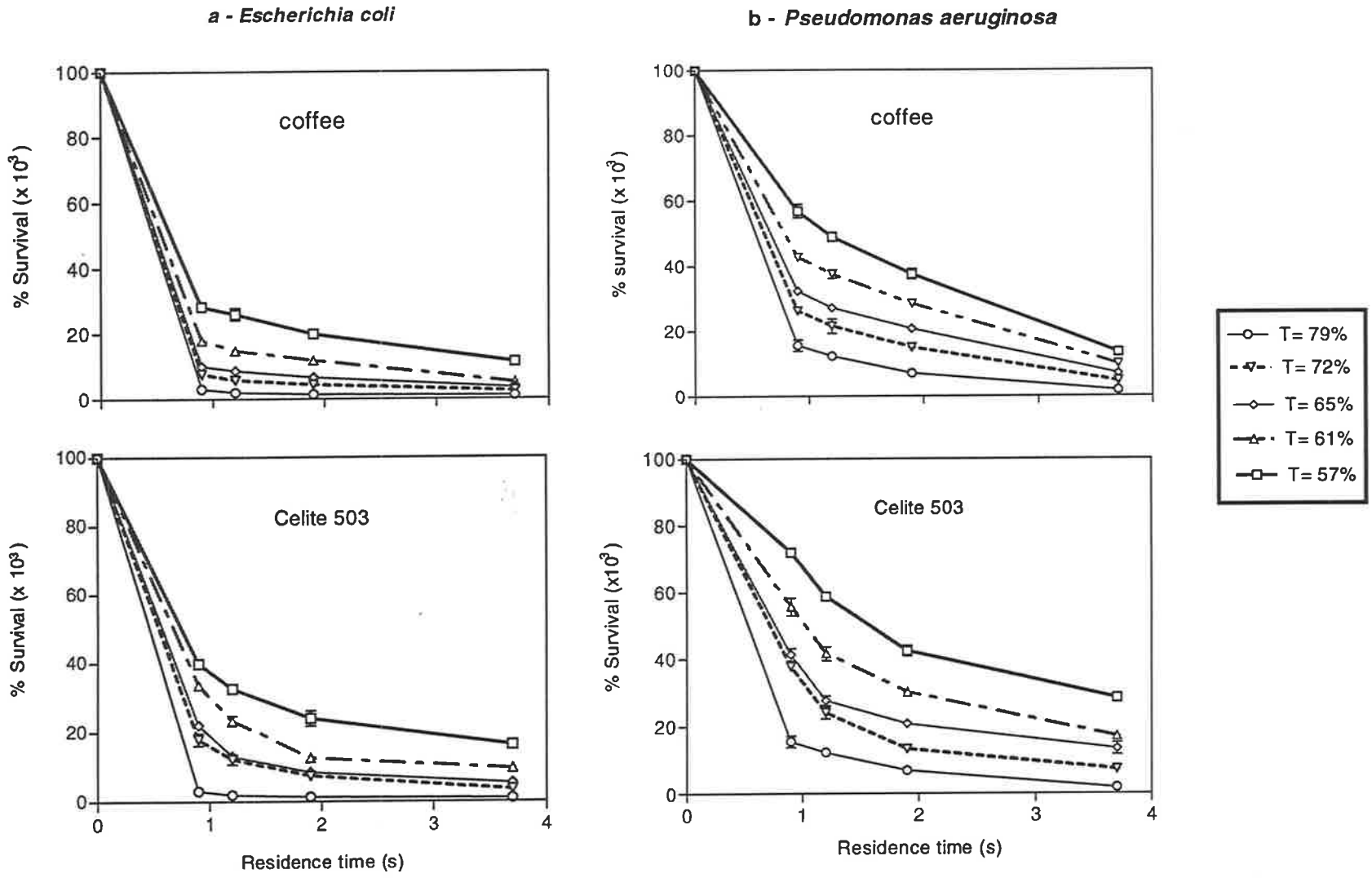


Figure 4.1 **Influence of residence time on survival of *E. coli* and *P. aeruginosa*. Feed-water UV transmittance (254 nm) was adjusted by addition of either coffee or Celite. Survival data are plotted for five different feed-water UV transmittance values**

Table 4.2 **Average UV dose applied by the experimental UV-LC5 disinfection unit as a function of feed-water flow rate and residence time of feed-water in the unit**

Feed-water Flow rate ($L.s^{-1}$)	Feed-water Residence time t (s)	Average UV dose, $D = It$(*) ($\mu Ws.cm^{-2}$)
0.0167	3.7	44,200
0.0333	1.9	22,700
0.0500	1.2	14,100
0.0667	0.9	10,800

* The LC5 disinfection unit lamp emits an average intensity of 11,940 μWcm^{-2} (UVTA, *pers. comm.*)

Table 4.3 **Feed-water residence time required for inactivation of suspensions of *E. coli* and *P. aeruginosa.* The feed-water UV transmittance (254 nm) was adjusted by addition of Celite**

Inactivation (%)	*E.coli*		*P.aeruginosa.*	
	UV transmittance (%)	Residence time (s)	UV transmittance (%)	Residence time (s)
99.99 %	52	3.7	52	4.7*
	60	2.3	60	3.7
	66	1.2	66	2.9
	70	0.4	70	1.9
99.98 %	52	1.9	-	-
	60	1.2	66	1.9
	66	0.5	70	1.2
99.97 %	52	1.2	52	3.2
	60	0.4	60	1.9
	-	-	66	0.9
99.95 %	-	-	52	1.2
	-	-	60	0.9

* by extrapolation of the experimental data

Table 4.4 **Effect of initial number of viable bacteria on the efficacy of UV inactivation. Feed-water UV transmittance (254 nm) was not adjusted by addition of UV absorbing agents or suspended solids**

Test micro-organism *	UV dose ($\mu Ws.cm^{-2}$)	Initial number ($number.mL^{-1}$)	Survivors ($number.mL^{-1}$)
E. coli	10,800	$< 5 \times 10^5$	0
E. coli	10,800	$< 8 \times 10^6$	100 - 200
E. coli	10,800	$> 5 \times 10^7$	500 - 3000
P. aeruginosa	10,800	$< 3 \times 10^4$	0
P. aeruginosa	10,800	$< 5 \times 10^5$	50 - 300
P. aeruginosa	10,800	$< 8 \times 10^6$	500 - 5000
P. aeruginosa	10,800	$> 5 \times 10^7$	1000 -10000

* No addition of absorbing agent (coffee) or suspended solids (Celite 503)

Figure 4.2 **Log_e survival *vs* UV dose for suspensions of *E. coli* and *P. aeruginosa* in feed-water. The feed-water UV transmittance (254 nm) was adjusted to 65% by addition of a UV absorbing agent (coffee) or suspended solids (Celite) prior to addition of the bacteria**

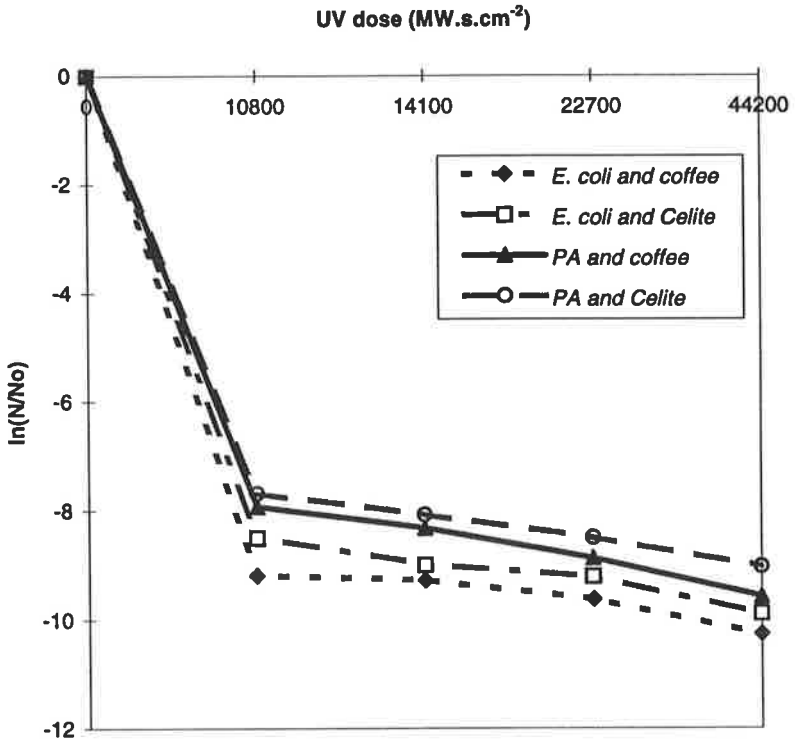
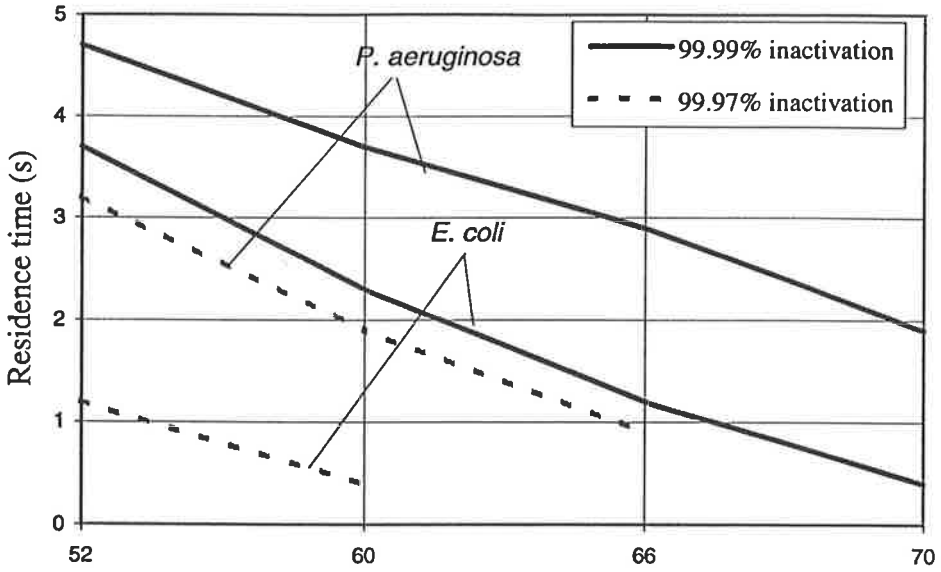


Figure 4.3 **Residence time required for inactivation of suspensions of *E. coli* and *P. aeruginosa*. The feed-water UV transmittance (254 nm) was adjusted by addition of suspended solids (as Celite) prior to addition of bacteria**



4.2 Effect of feed-water UV transmittance on survival

4.2.1 Effect of UV transmittance

Throughout the experimental work described in this thesis, feed-water UV transmittance (at 254 nm) was adjusted to values between 50 and 80% by addition of a UV absorbing agent (coffee) or suspended solids (Celite). Overall, survival of the two test strains showed similar responses to changes in feed-water UV transmittance following exposure to UV light from the disinfection unit (Figure. 4.4). Results obtained indicate that the % survival (100 x N/N_0) and log survival ratio (as $\ln N/N_0$) of suspensions of both *E. coli* and *P. aeruginosa* increased with decreasing UV transmittance. When expressed in terms of inactivation, the efficacy is reduced as UV transmittance decreases (or absorbance/turbidity increases). Quantitatively, the inactivation efficacy increased by a factor of 5 to 10 as feed-water UV transmittance was increased from 50 to 80%.

Thus in the presence of only low concentrations of coffee and Celite, there is less absorption and scattering of the UV light respectively, and therefore the efficacy of inactivation of bacterial cells by UV irradiation is greater. This is in agreement with other studies (Job *et al.* 1995; Qualls *et al.* 1985). However, Wolf *et al.* (1979) came to the surprisingly different conclusion that no significant relationship existed between suspended solids concentration and the degree of inactivation.

4.2.2 Effect of absorbing agent and suspended solid on survival

UV Absorbing agents and suspended solids have quite different effects on UV transmittance. UV absorbing agents affect UV inactivation by effectively absorbing UV light before it has a chance to interact with bacteria. Suspended solids, however, shield bacteria from the harmful effects of UV irradiation. The higher the concentration of suspended solids, the fewer lethal hits on bacteria in suspension. As can be seen in Figure 4.4, at equivalent values of UV transmittance, survival of test bacteria in the presence of Celite is greater than observed for feed-water containing coffee. For example, with a mid-range of residence time of 1.9 s and at 55% UV transmittance (ie 0.001 $g.L^{-1}$ coffee and 0.01 $g.L^{-1}$ Celite 503) % survival of *E. coli* is 0.0043 and 0.0074 respectively.

The difference between the survival of *E. coli* and *P. aeruginosa* cells in the presence of coffee and Celite 503, is probably due to differences in typical physical properties of the materials. For example, diatomaceous earth as Celite 503 particles of median size 23 μm, will limit UV disinfection by scattering of the UV light, and by shielding cells from the damaging effects of UV light. Coffee, because it contains a wide range of complex organic molecules with conjugated bonds, absorbs UV light to an extent that is dependent on concentration and UV dose. Other colloidal and chemical properties of additives to populations of bacterial cells in water may also be important in influencing survival during and after UV exposure. However, since Celite 503 is uncharged, it is highly unlikely that *E. coli* cells, which are negatively charged, will adsorb to Celite particles by either electrostatic or electrical double layer phenomena. Nevertheless, this aspect is worthy of further examination.

The Ultraviolet - visible spectra of coffee and Celite 503 solutions at different concentration shows that coffee absorbs UV light much greater than Celite 503. This can be seen in Figure 4.5 and Appendix G.

Figure 4.4 **Survival (100 x N/N_0) of test bacteria suspended in feed-water and exposed to a UV dose of 22,700 $\mu Ws.cm^{-2}$. The feed-water UV transmittance (254 nm) was adjusted by addition of known concentrations of coffee or Celite prior to addition of bacteria**

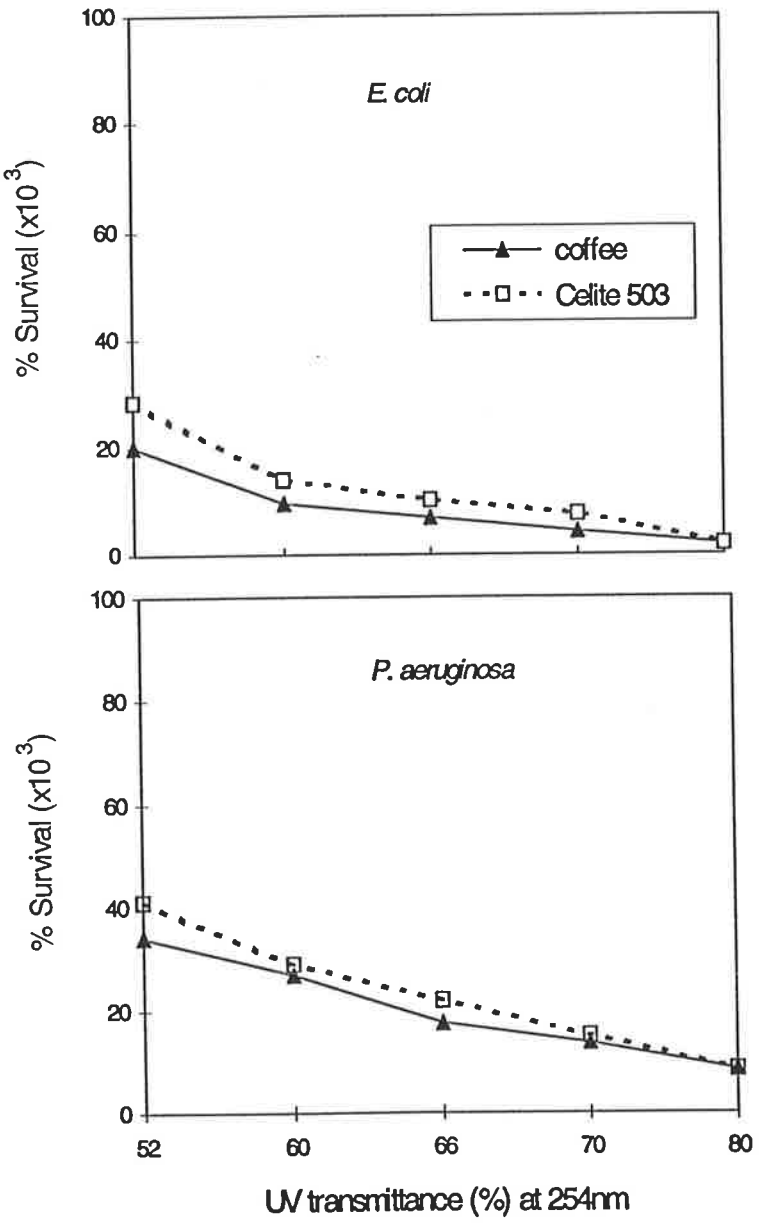
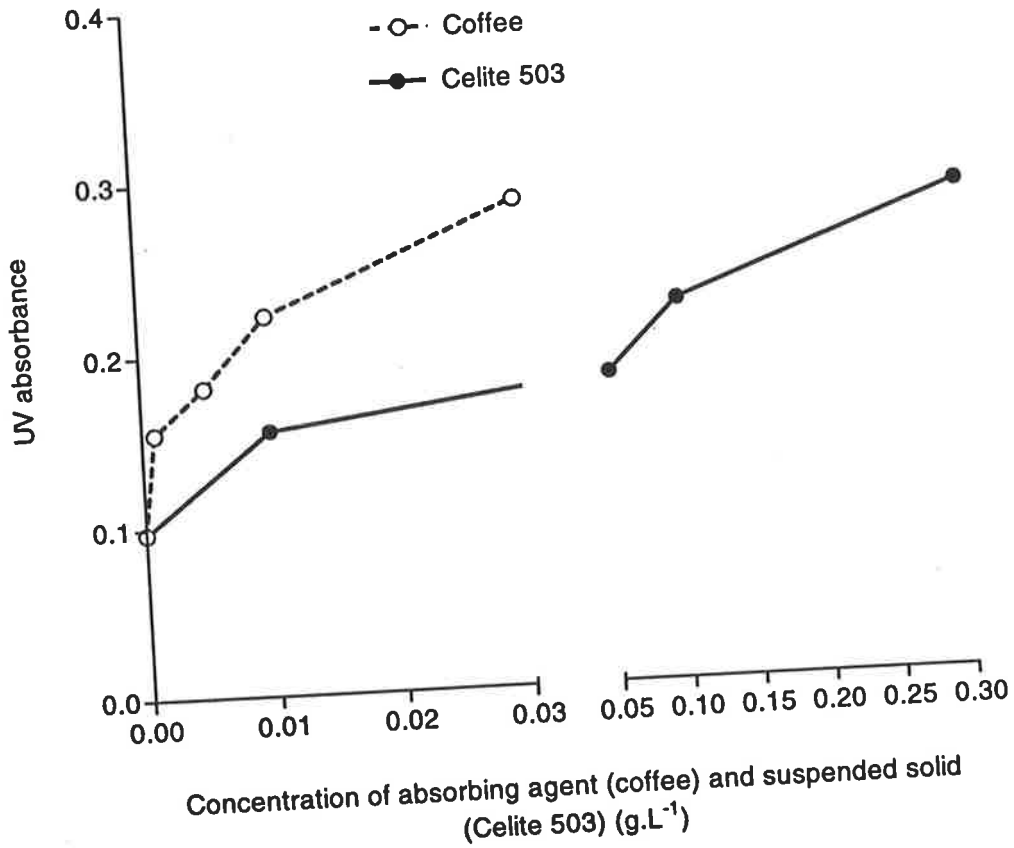


Figure 4.5 **Relationship between UV absorption and concentration of UV absorbing agent (coffee) and suspended solids (Celite 503) added to feed-water**



4.3 Bacterial re-growth following UV exposure

An important part of any study of UV disinfection is a confirmation that the quality of disinfected water is maintained during subsequent storage. The presence of inactivated and sub-lethally injured bacteria present within treated potable water would potentially represent a significant public health issue. Clearly, resuscitation and repair of sub-lethally injured bacteria could result in re-establishment of a viable population. It is possible that in the presence of nutrients released from inactivated cells, that resuscitation of sub-lethally injured cells could take place. This process could be promoted by light or dark repair mechanisms known to be responsible for correcting UV induced damage to DNA. However, cryptic growth of bacteria which survive UV disinfection may also be an important issue concerning the quality of stored disinfected water. Nutrients released from inactivated cells may be sufficient to promote growth and replication of survivors, or at the very least, provide a source of maintenance energy.

In view of these issues, the quality of stored, disinfected water was investigated by monitoring numbers of bacteria in volumes of treated water incubated at room temperature in either light or dark incubation environments. After defined periods of incubation, the treated suspensions were plated on NA and counts of bacteria compared to those obtained immediately following UV treatment. Data shown in Figure 4.6 expresses the change in number of survivors as a regrowth ratio (N_{rp}/N_0) ie. the number of viable bacteria after storage compared with the viable count obtained immediately following UV irradiation. The re-growth ratio of both *E. coli* and *P. aeruginosa* in stored water, incubated on the laboratory bench, increased markedly with increased storage time (Figure 4.7). At UV dose of 22,700 $\mu Ws.cm^{-2}$ (exposure time 1.9 s) the re-growth ratio increased from 0.000357 to 0.221000 for *E. coli* and from 0.000022 to 0.0025 for *P. aeruginosa*, respectively, after 24 and 144 h storage at 37°C with light (Figure 4.7).

Furthermore, the re-growth ratio following UV exposure of both *E. coli* and *P. aeruginosa* decreased with increased residence time in the UV disinfection unit. For example, using residence times of 0.9 and 3.7 s respectively, re-growth ratios obtained for *P. aeruginosa* suspensions were 0.00480 and 0.000155 after 24 h; 0.00268 and 0.00086 after 72 h; 0.0068 and 00.00050 after 144 h when treated suspensions were stored at room

temperature. Similarly, for *E. coli* the re-growth ratios were 0.00082 and 0.000128 after 24 h; 0.00745 and 0.00568 after 72 h, and; 0.0717 and 0.041 after 144 h.

Figure 4.7 also indicates a possible effect of storage temperature on re-growth. When stored at 37C, re-growth was greater than that observed for treated water stored at 30C. Conversely, no significant effect of light or dark storage conditions on re-growth was observed (Figure 4.7). Nevertheless, re-growth was not detected until 72 h post disinfection, irrespective of the storage temperatures used or storage in the presence or absence of light.

These effects have been observed previously (Cains, 1993; Chen *et al.*1993). Even with short storage times (4 h) following UV exposure, re-growth has been shown to result in a 1 log unit increase in numbers of coliforms and a 0.7 log unit increase in the Standard Plate Count of treated water (Chrtek & Popp, 1991). Similarly, Mechsner *et al.* (1991) concluded marginally higher numbers of *E. coli* cells are obtained following re-growth than the period prior to treatment. However, it was observed that the number of *E. coli* declined after 6 days storage under light and remained effectively constant in the dark.

Re-growth of *E. coli* cells in the suspensions reached nearly 25 % of the pre-treated levels after 6 days storage, whereas re-growth of *P. aeruginosa*. Reached only approximately 0.2 % of the initial bacterial densities. The significantly high re-growth ratio for *E. coli* is in agreement with previous findings of Mechsner *et al.* (1991) who reported re-growth of the injured population reached nearly 30% of the pre-exposure level.

No significant effect of UV transmittance (either in the presence of absorbing agent, as coffee, or suspended solids, as Celite 503) on the re-growth ratio of both test micro-organisms was observed, especially after short periods of storage (Figure 4.8). More complete data are needed to determine the significance of the presence of UV absorbing agents and suspended solids on survival and re-growth.

These results show re-growth of bacteria following UV disinfection needs to be accounted for in any study of UV disinfection efficacy. However, the results presented do not indicate whether cryptic growth or DNA repair mechanisms are responsible for the effects observed.

Figure 4.6 **Regrowth ratio (N_{rp}/N_0) of a) *E. coli* and b) *P. aeruginosa* after UV exposure versus residence time (stored at room temperature, 24°C)**

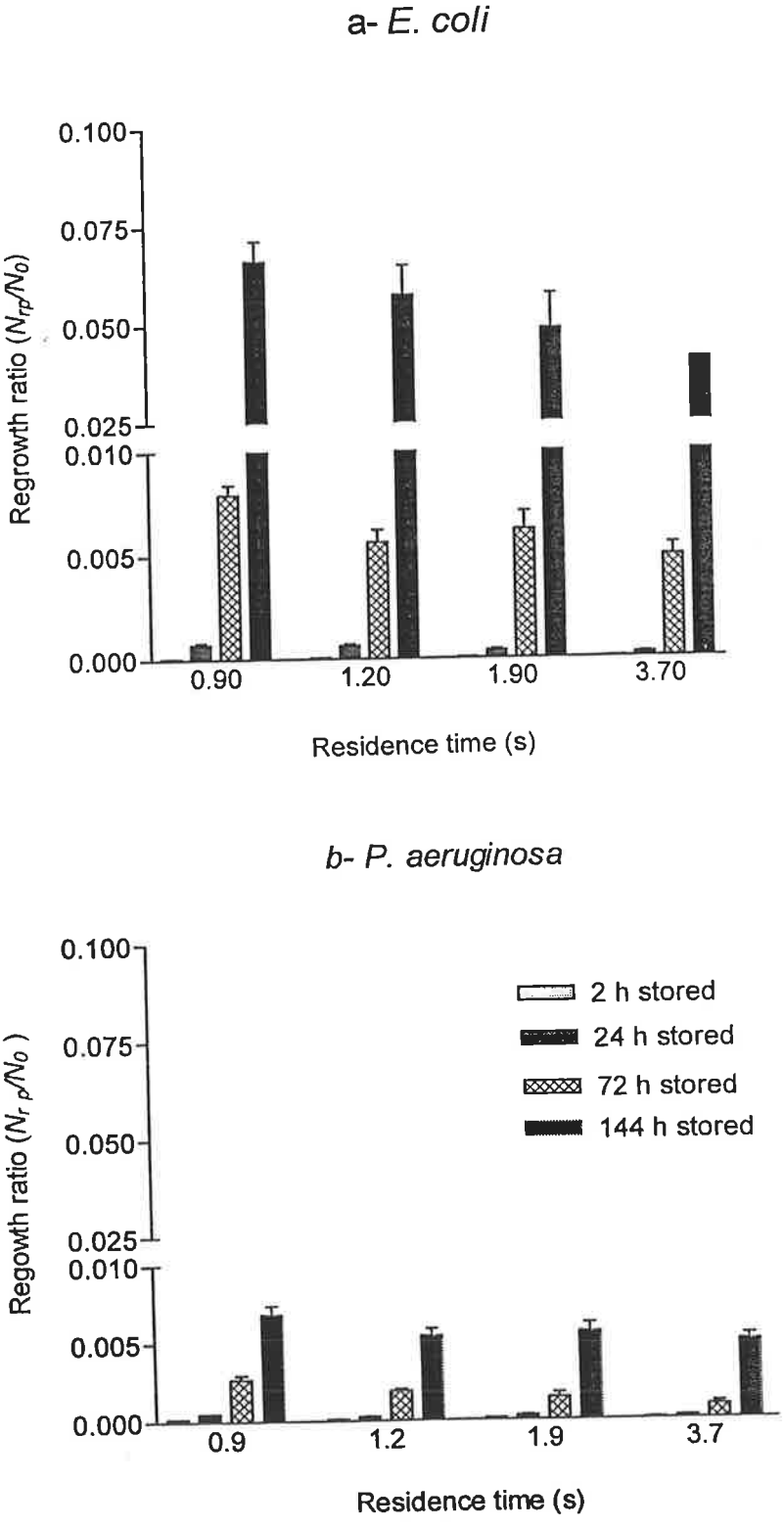


Figure 4.7 **Effect of storage time on the re-growth of a) *E. coli* and b) *P. aeruginosa* after exposure to UV dose of 22,700 μWs.cm^{-2} under different storage conditions**

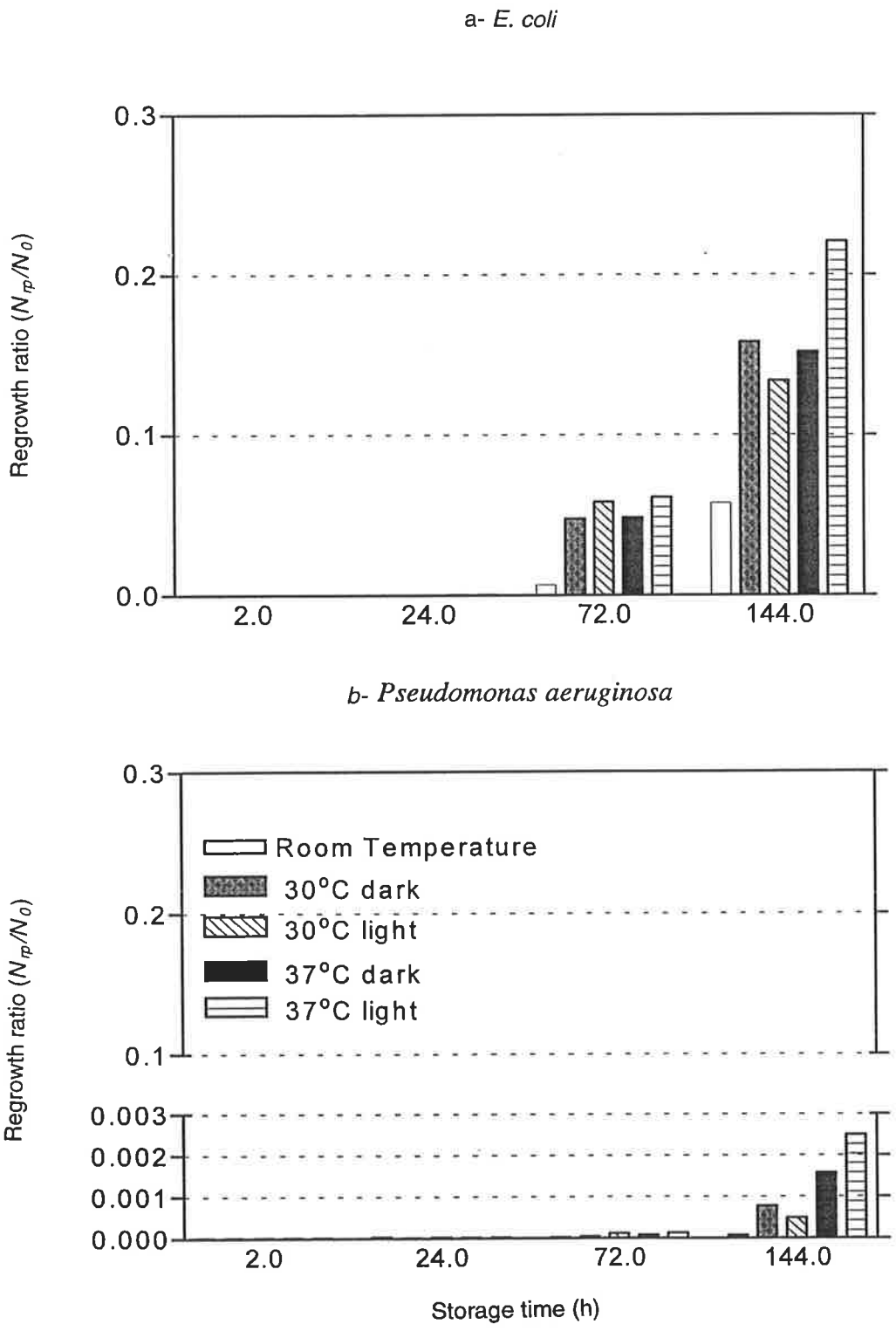
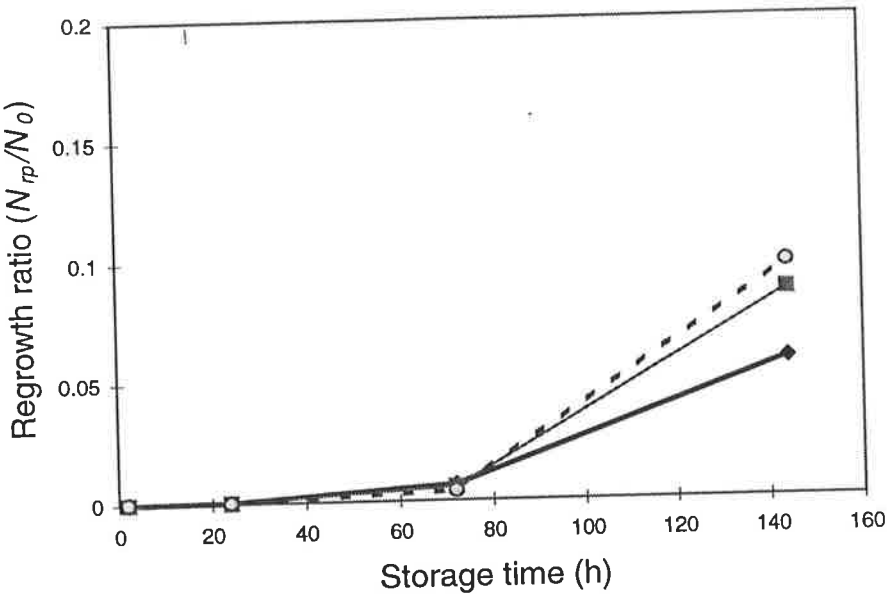
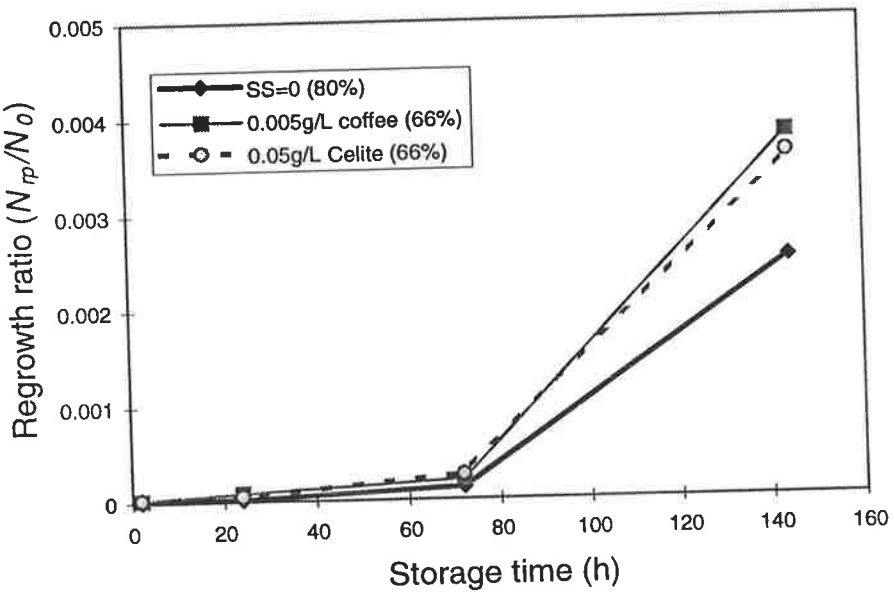


Figure 4.8 **Effect of absorbing agent (coffee) and suspended solids (Celite 503) on the re-growth of a) *E. coli* and b) *P. aeruginosa* after exposure to UV dose of 22,700 μWs.cm^2 (stored at 37°C under light)**

a- *E. coli*



b- *P.aeruginosa*



4.4 Model synthesis for UV inactivation kinetics

An important aim of this study is the synthesis of appropriate UV inactivation kinetics for disinfection of the test bacterial contaminants. Synthesis involved all 260 data sets (Table 4.1 and replicates Appendix F). These were regressed using linear regression (Snedecor & Cochran, 1969) undertaken conveniently in spreadsheet form using Excel™ for each combination of test micro-organism and concentration of adsorbing agent (coffee) and suspended solids (Celite). Initial data analysis strongly suggested linear, first-order kinetics as is illustrated in Figure 4.2.

4.4.1 Regression analyses

The regression analysis is conveniently summarised in Table 4.5. It is seen from the table that with different combinations of absorbing agent and SS the correlation coefficient (R^2) ranged from 0.87 to 0.98 - with an overall mean of $R^2 = 0.89$. The value for *E. coli* with no additions of absorbing agent or SS (line 1 Table 4.5) of $R^2 = 0.51$ is very much less than the overall mean value (0.89) and is not readily explained.

However, the overall high values of R^2 strongly suggest highly linear, or first-order, reaction kinetics (as expressed by equation 2.6) for the disinfection of suspensions of both *E. coli* and *P. aeruginosa.*

4.4.2 Analyses of residual plots

As an analysis of residuals (ie observed value - predicted value) is critical to an appraisal of a model, residual plots were investigated. These are best presented as predicted value *vs* observed value of the reduction in viable cell numbers with differing UV dose.

The residual plot for *E. coli* suspension with a mid-range value of concentration of absorbing agent (coffee) of 0.01 gL^{-1} is given as Figure 4.9a. That for *P. aeruginosa* in the presence of 0.1 gL^{-1} Celite is given as Figure 4.9b. The plots show residuals evenly distributed with no apparent structure to them. This strongly supports the first-order disinfection model for inactivation kinetics as being highly appropriate.

The residuals for all the 260 data sets were examined. These are presented in Figures 4.10a and 4.10b, respectively, for *E. coli* and *P. aeruginosa*. Clearly, there is no structure to the residuals over all the data sets.

It is clear therefore that the disinfection kinetics of suspensions of bacterial cells of both *E. coli* and *P. aeruginosa* are therefore highly first-order. Equation 2.6 is a highly appropriate model form.

4.4.3 Extrapolation of the model for disinfection kinetics

It must be cautioned that extrapolation of the model outside the range of experimental values of UV dose, UV transmittance and concentrations of both absorbing agent and SS used in the synthesis of the model, must be done carefully. This is especially true for UV doses below 10,800 $\mu W.s.cm^{-2}$. The reason is seen readily from inspection of Figure 4.2 where a significant change in slope must occur to permit a, zero UV dose-zero reduction of viable cell numbers ($\ln N/N_o$), intercept.

4.4.4 Using the kinetic model for UV inactivation

The prediction of the efficacy of UV disinfection of suspensions of both test micro-organisms can be readily and confidently made using the data of Table 4.5.

For example, for *E. coli* with 0.01 gL^{-1} absorbing agent (coffee), the model for UV disinfection with UV dose is given by:

$$\ln(N/N_0) = -3.4 \times 10^{-5} D - 8.82 \qquad (4.1)$$
$$(R^2 = 0.91)$$

Predictions from equation 4.1 and a comparison with observed data is made in Table 4.6. It is apparent the model gives a very good fit to observed data and there is good spread of the residuals. Similarly, other models can be obtained from Table 4.5 for a range of combinations of absorbing agent and SS for suspensions of both test micro-organisms. Figure 4.11 presents a graphical summary of the inactivation rate plotted as $\ln(kI)$. The data show smooth linear curves as would be expected.

Table 4.5 **Summary of regressions of UV disinfection data (as $\ln N/N_0$ *vs* UV dose *D*) for *E. coli* and *P. aeruginosa* with combinations of concentrations of absorbing agent and suspended solids**

Micro-organism	n*	AA or SS ** gL^{-1}	*k* s^{-1}	Intercept	Correlation coefficient, R^2
E. coli	12	nil	-2.7 E-5	-10.38	0.51
E. coli	12	0.001 coffee	-3.1 E-5	-9.26	0.87
E. coli	16	0.005 coffee	-3.1 E -5	-8.93	0.87
E. coli	12	0.0 1 coffee	-3.4 E-5	-8.42	0.91
E. coli	12	0.03 coffee	-2.5 E-5	-7.98	0.93
E. coli	12	0.01 Celite	-4.6 E-5	-8.31	0.87
E. coli	16	0.05 Celite	-3.8 E-5	-8.28	0.81
E. coli	12	0. 1 Celite	-3.2 E-5	-7.86	0.80
E. coli	12	0.3 Celite	-2.5 E-5	-7.64	0.90
P. aeruginosa	16	nil	-6.5 E-5	-8.11	0.96
P. aeruginosa	16	0.001 coffee	-5.2 E-5	-7.71	0.95
P. aeruginosa	16	0.005 coffee	-4.6 E-5	-7.52	0.97
P. aeruginosa	16	0.0 1 coffee	-4.5 E-5	-7.24	0.98
P. aeruginosa	16	0.0 3 coffee	-4.3 E-5	-6.98	0.98
P. aeruginosa	16	0.01 Celite	-4.6 E-5	-7.63	0.86
P. aeruginosa	16	0.05 Celite	-3.1 E-5	-7.65	0.81
P. aeruginosa	16	0. 1 Celite	-3.3 E-5	-7.26	0.93
P. aeruginosa	16	0.3 Celite	-2.6 E-5	-7.05	0.93

*. number of data sets

**AA = absorbing agent (coffee); SS = suspended solids (Celite 503)

Table 4.6 **Predictions for UV disinfection of a suspension of viable *E. coli* cells in the presence of 0.01 gL^{-1} absorbing agent (coffee) (UV transmittance = 60%)**

observed $\ln(N/N_o)$	predicted $\ln(N/N_o)$	residual
-9.90	-9.91	+0.00932
-9.25	-9.19	-0.06458
-9.13	-8.89	-0.23728
-8.87	-8.78	-0.08924
-9.85	-9.91	+0.06759
-9.00	-9.19	+0.19136
-8.84	-8.89	+0.05732
-8.61	-8.78	+0.17860
-9.94	-9.91	-0.03150
-9.36	-9.19	-0.17458
-9.00	-8.90	-0.10723
-8.58	-8.79	+0.02002

Figure 4.9 **Predicted value of $\ln(N/N_o)$ *vs* observed value of $\ln(N/N_o)$ at a UV transmittance of 60% for a) *E. coli* with 0.01 gL^{-1} absorbing agent (coffee) b) *P. aeruginosa* with 0.1 gL^{-1} suspended solids (Celite 503)**

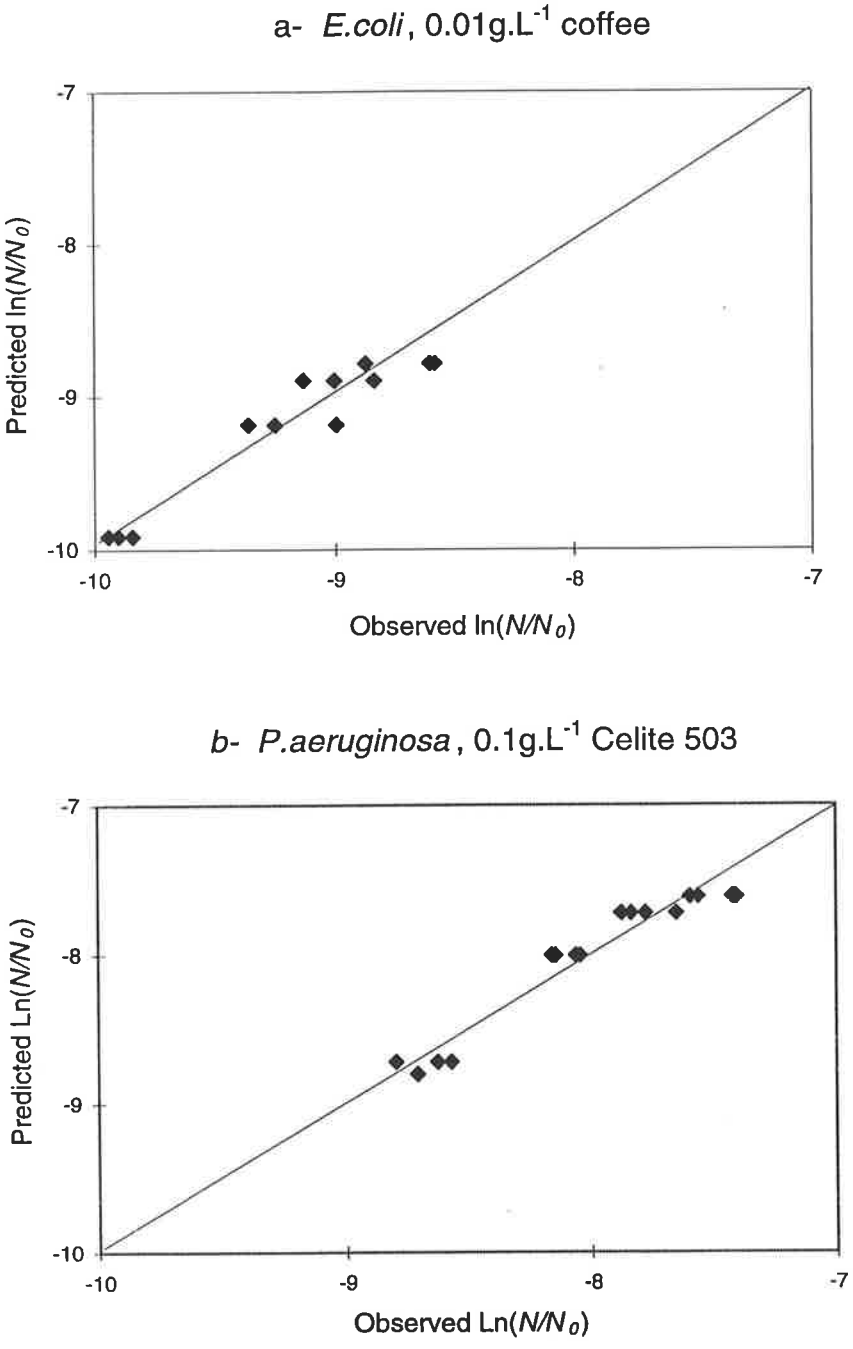


Figure 4.10 **Predicted value of $\ln(N/N_o)$ *vs* observed value of $\ln(N/N_o)$ in the presence of both absorbing agent (coffee) and suspended solids (Celite 503) for all disinfection data for a) *E. coli* and b) *P. aeruginosa***

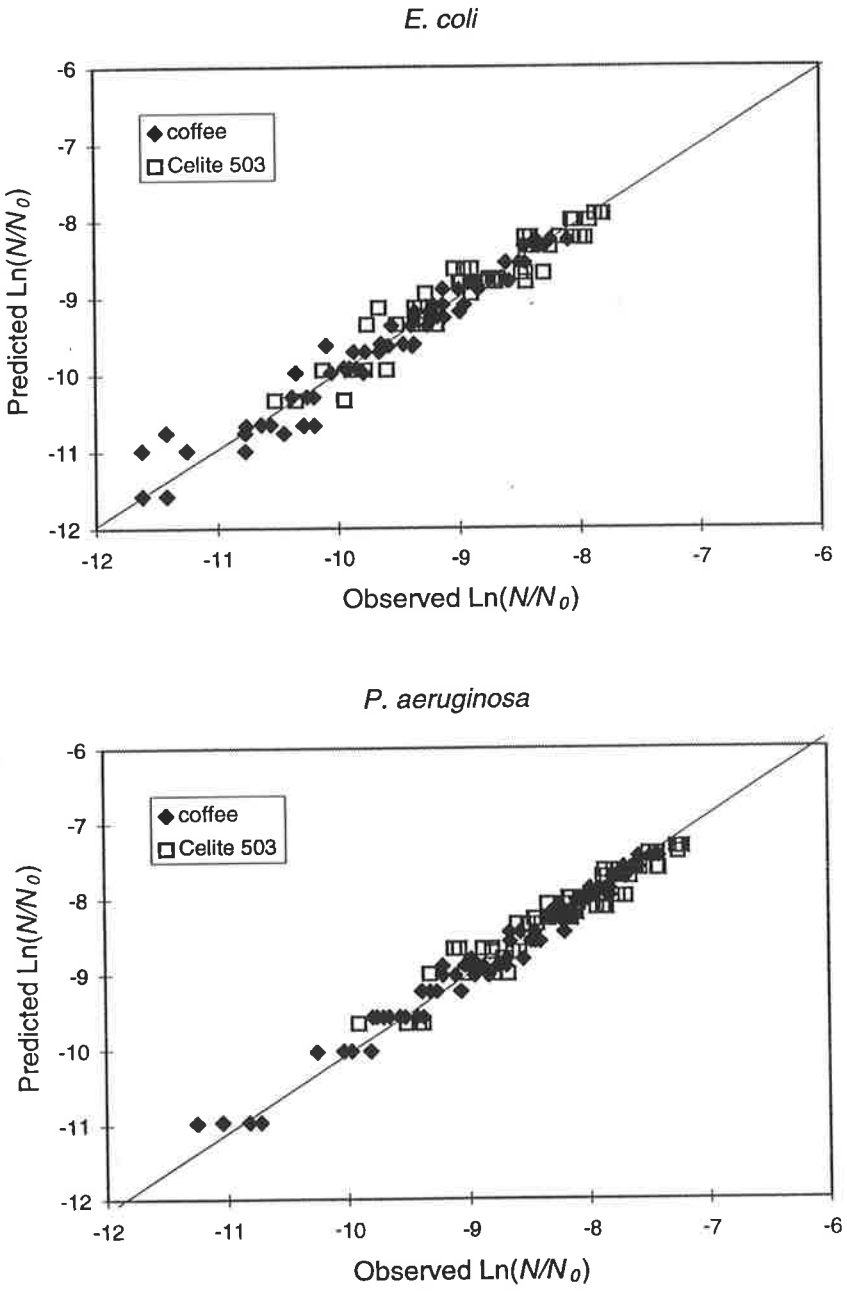
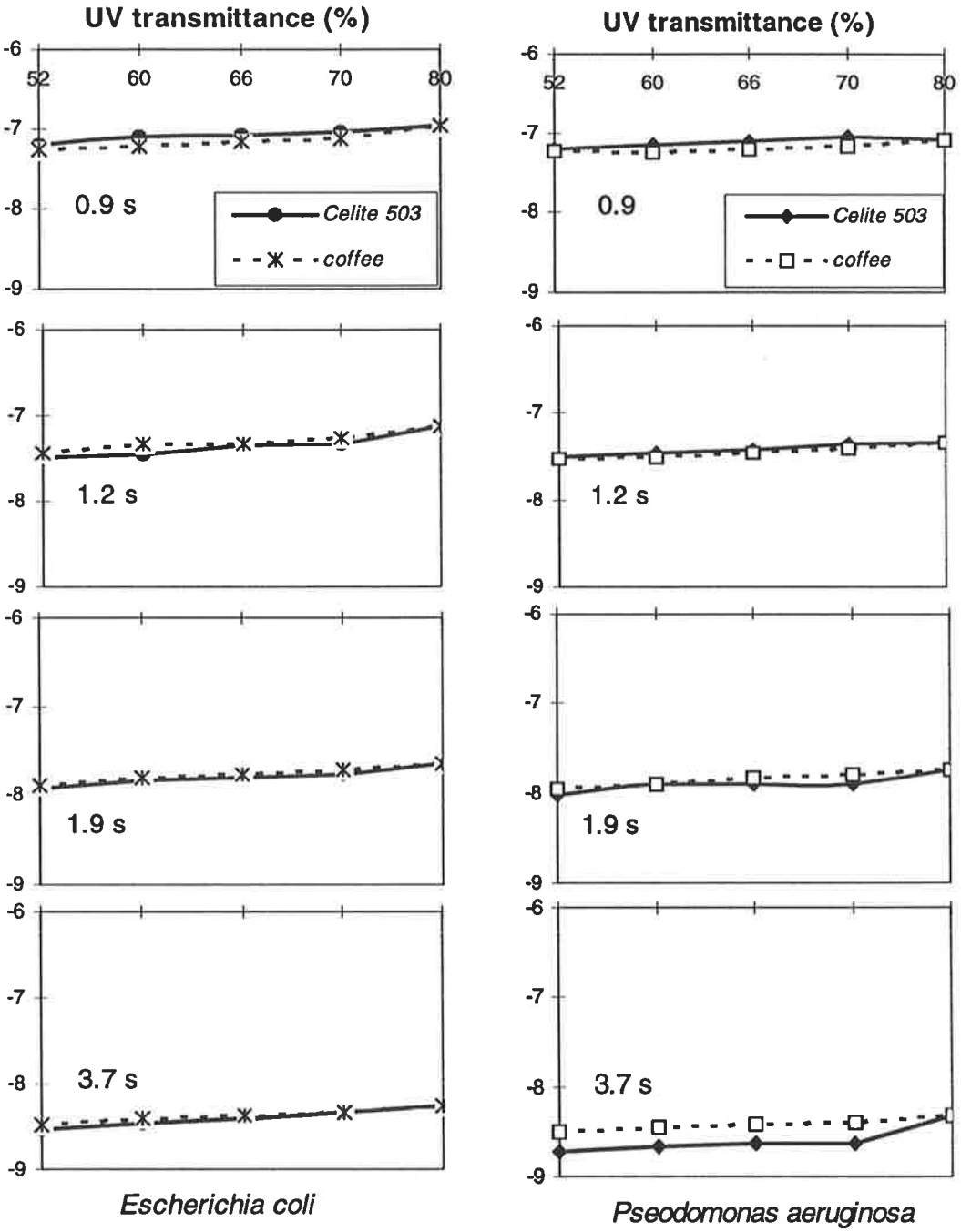


Figure 4.11 Effect of UV transmittance (254 nm) on inactivation rate (ln*kI*) of *E. coli* and *P. aeruginosa* at various residence times



4.5 Other observations

The data of Table 4.4 appears to show that when the number of initial test micro-organisms is greater there is a greater number of cells surviving UV disinfection. This behaviour might be explained in part by a small fraction of the viable cell population being more resistant to UV light than the majority of the cell population. That is, a distribution of resistance to UV inactivation within in the viable cell population, might account for this finding. Alternatively, at low viable cell populations, suspended solids in the water may shield the remaining small number of cells from UV light, extending their survival.

However the point has been made that the (dimensionless) rate of inactivation is not influenced by initial cell numbers.

4.6 Summary of major experimental findings and a comparison with findings of previous work

- The UV dose required to inactivate of *E. coli* was greater than that reported by a number of researchers in the literature, for example, Jevons (1992) and Crandall (1986). However, our study showed similar results with those reported for example by Trojan Technologies, Inc.(1990) in that for a 4-$\log_{10}$ inactivation (99.99%) of a faecal coliform concentration, a UV dose of approximately of 30,000 $\mu Ws.cm^{-2}$ is required at water transmittance of 65% - this compares well with the value of the present study of 14,100 to 22,700 $\mu Ws.cm^{-2}$.

- Suspensions of both test micro-organisms of *E. coli* and *P. aeruginosa* showed similar "survival *vs* residence time" relationships with UV radiation in the presence of UV absorbing agent (coffee) and suspended solids (Celite 503). Bacterial cells associated with SS particles were partially shielded from UV inactivation. The protection of these cells is one factor limiting improved UV disinfection.

It is unclear whether there is a correlation between UV transmittance and the ratio of the photo-repaired to non-photo-repaired faecal and total coliforms after UV disinfection. A finding supported by Whitby & Palmateer (1993).

- Similar inactivation efficacy responses were observed in the suspensions of the test micro-organisms to absorbing agent and suspended solids. The UV dose or number of UV lamps required increased as water transmittance decreased. For example, for a 99.99% inactivation of a suspension of *E. coli* with 66% transmittance, a UV dose of 14,100 $\mu Ws.cm^{-2}$ was required but at a transmittance of 52%, 44,200 $\mu Ws.cm^{-2}$ was required. Mann and Cramer (1992) report similar quantitative findings for faecal coliforms in that at a UV transmittance of 50% double the UV dose was required compared with that at a UV transmittance of 65%.

- In this study the re-growth of *P. aeruginosa,* and particularly of *E. coli* following UV exposure appeared significant. Re-growth of *E. coli* after 6 days storage reached nearly 25 % of the initial numbers of viable bacteria. This is in agreement with the results from the study of Mechsner *et al.* 1991. However they supposed that after 10 days storage the number of bacterial cells was decreased. Similar responses were reported by Hengesbach *et al.* (1993).

- Inactivation data show highly linear, or first-order, disinfection kinetics with UV dose for both test micro-organisms in different combinations of absorbing agent (coffee) and suspended solids. Venosa (1983) reported first-order kinetics of UV disinfection for a pure culture population of faecal coliforms.

- In the present study we have produced a significantly greater number of reliable data sets than has been reported in the literature.

CHAPTER 5 - CONCLUSIONS

1. Neither a UV light absorbing agent (as coffee) or a UV light scattering agent (as suspended solids of Celite 503) in the range of concentrations of, respectively, 0 to 0.03 gL^{-1} and 0 to 0.3 gL^{-1}, had a significant biocidal effect on viable cells of suspensions of *E. coli* or *P. aeruginosa*.

2. There was no significant adsorption of numbers of viable cells of either *E. coli* or *P. aeruginosa* to Celite particles.

3. Reductions in viable cell numbers of suspensions of both test micro-organisms were wholly attributable to UV dose in the UV-LC5 disinfection unit.

4. UV light absorbing agents (coffee) and scattering agents (Celite 503) have a significant influence on the efficacy of UV inactivation of viable cells of *E. coli* and *P. aeruginosa*.

5. In the presence of suspended solids (Celite 503) the efficacy of UV inactivation is limited significantly more so than that in the presence of absorbing agents (coffee).

6. Suspensions of viable cells of *P. aeruginosa* are more resistant to UV light than are those of *E. coli*.

7. Greater re-growth and repair of viable cells of *E. coli* after UV disinfection was observed with both light and dark storage conditions than with *P. aeruginosa*.

8. UV inactivation kinetics observed for both *E. coli* and *P. aeruginosa* in the presence of absorbing agent (coffee) and suspended solids are highly appropriately described by first-order kinetics.

RECOMMENDATIONS FOR FURTHER STUDY

- The mechanism of shielding or protecting viable bacterial cells by the particles of suspended solids should be further studied with the aim of a quantitative result. For example, is there any significant correlation of the number of surviving bacterial cells with concentration of suspended solid particles, and; what is the effect of size of suspended solids particles and electrical charge. Clay colloids might well protect bacteria from UV inactivation by adsorption of the negatively charged bacteria to positively charged colloids or adsorption of negatively charged bacteria to negatively charged colloids (electrical double layer phenomena).

- Re-growth kinetics of post-disinfected bacteria need to be better understood and the role of cryptic growth needs to be investigated.

APPENDIX A - A definition of some important terms used in this study

clumping	micro-organisms associate together to form a clump
disinfect	cleanse of infection
exposure time	residence time in UV unit
intensity	energy density of UV irradiation
irradiate	shine UV light upon
macromolecules	the main chemical components of cells
micro-organism	a large diverse group of organisms that exist as single cells or cell clusters
nutrient broth	solution in which grow micro-organisms
pathogen	micro-organism causing disease to humans
potable	drinkable
photo-reactivation	repair system of bacteria injured by UV light
residence time	time for which an element of fluid is in the UV unit
re-growth	micro-organisms growth following UV exposure
repair	recovery of damaged micro-organisms after UV exposure
shielding	physical barrier to UV transmittance protecting bacterial cells
transmittance	indicates absorption of energy per unit depth of the water
turbidity	how muddy, thick, unclear a solution is
tubulator	static mixer
ultraviolet	invisible rays of the spectrum beyond the violet rays
viable	capable of maintaining life
viable counts	a measure of the concentration of 'living' micro-organisms

APPENDIX B - Disinfection methods used in some countries (adapted from Bitton, 1994)

Methods	Cl_2	ClO_2	$ClNH_2$	O_3	UV
Australia	+++	+	++		+
Austria	+++	+		+	+
Belgium	+++	+		+	
Bulgaria	+++				+
P R China	+++			+	
Czech Republic	+++			+	
Finland	+++	+	+	+	
France	++	++		++	
Germany	+++	+++		++	+
Hungary	+++		+	+	
Ireland	+++			+	
Italy	+++	+++			
Japan	+++				
Macao	+++				
Netherlands	+			+	+
Norway	++		+		++
South Africa	+++		+	+	
Spain	+++	+		++	
Sweden	+++	+	++		
Switzerland	+	++		++	++
UK	+++	+	+		+
USA	+++	+	+	+	+

+++ Dominating method

++ Practised commonly

+ Practised occasionally

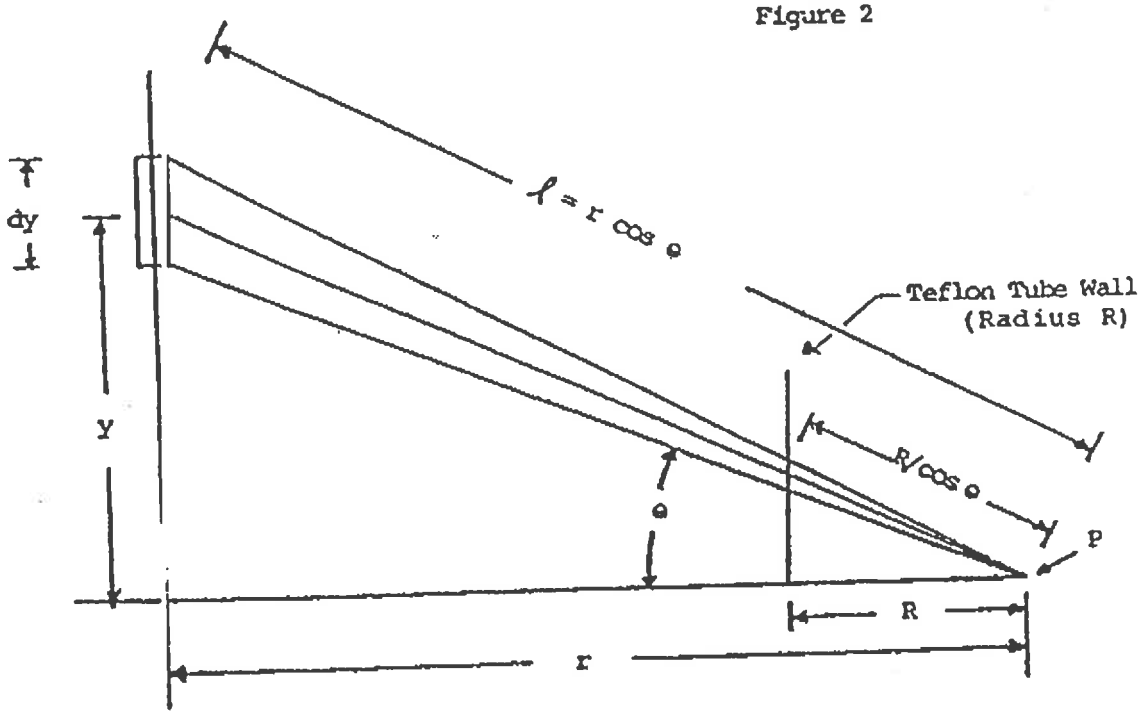
APPENDIX C - Calculation of UV intensity

Ultraviolet Intensity

The point of minimum intensity in the UTI system is at the center of each Teflon tube. Ultraviolet light is emitted from the outer surface of each lamp and is decreased in intensity as it penetrates to the center of the tube by geometrical factors and absorption by the Teflon wall and by the fluid contained in the Teflon tube.

Intensity is calculated by the following two dimensional mathematical model. Figure 2 depicts a point, "P", which is receiving light from a cylindrical source of width, "W", and length "Y". Point "P" is located at a distance 'r' from the center of the source and receives light from many differential elements of the source in which the light path is a distance of r cos θ.

Figure 2



Three optical parameters need to be defined:

1. E = Source radiance (watts/steridian $-cm^2$) which is constant for a lambertian source
2. I_θ = Source intensity, watts/steridian
3. E_s = Scalar irradiance at point "P", watts/cm^2, (which corresponds to the intensity of Equation 1) Es is the scalar product of light vectors from all directions that impinge on point "P".

The assumptions made in developing this simplified model for hand calculation are listed below:

1. No ultraviolet light is absorbed in the air space between the source and the Teflon tube. According to Koller (4), air absorption can be neglected if the light path is less than one meter.
2. In the two dimensional model, the light absorbance distance in the water is assumed to be the tube radius. This assumption is conservative because much of the light travels a shorter path length through the tube cross-section.
3. Index of refraction changes from air to the Teflon tube and from the tube to water are neglected. The indices of refraction are: air, 1.0; Teflon, 1.34; water, 1.33. As light travels from air to Teflon there will be a deflection of the rays that will tend to yield a higher intensity. The indices of refraction of Teflon and water are so close that there is a negligible effect.
4. Scattered light (path radiance) is considered to be totally absorbed and is included in the Teflon and water absorption coefficients. Actually, scatterd light is not absorbed and may be useful for disinfection. This introduces additional conservatism into the mathematical model.
5. Surface reflections from the Teflon tube are included in transmittance of the wall.
6. Transmission of light through the Teflon tube wall is constant with angle of incidence up to an angle of 60° and is to be zero at higher angles. Experimental measurements by E.I. DuPont indicate that Teflon efficiently transmitts UV light at incidence angles up to 85°.
7. UV light is attenuated in the water according to Bouger's (sometimes called Lambert's) law and the path length is given by the tube radius divided by the cosine of the angle of the individual light paths. $I_{\theta}/I_{o} = e^{-\frac{\alpha R}{\cos \theta}}$, where α is the absorption coefficient.
8. Turbulence is accounted for by computing the area average intensity base on a path length equal to the tube radius. Actually, a smaller path

length should be used, which would yield a higher calculated scalar irradiance.

Differential scalar irradiance at point "P" is given by

$$dE_s = \frac{dI_o}{l^2} \qquad (2)$$

which is equal to

$$dE_s = \left(\frac{w\epsilon \cos\theta}{r}\right)(T)\left(e^{-\frac{\alpha R}{\cos\theta}}\right) d\theta \qquad (3)$$

where T is the transmittance of the Teflon tube wall.

Integrating over an angle of plus or minus 60^o ($\pi/3$) yields

$$E_s = \frac{wBT}{r} \int_{-\pi/3}^{\pi/3} \cos\theta\, e^{-\frac{\alpha R}{\cos\theta}}\, d\theta \qquad (4)$$

The above integral could not be solved by conventional mathematical techniques, so the integral was evaluated by numerical integration for a range of values of αR likely to be encountered in water disinfection. Figure 3 indicates the results of the numerical integration. For the special case of zero absorption coefficient, the value of the integral is the square root of three.

To account for the presence of turbulence in the two-dimensional model, the area - weighted average scalar irradiance was determined.

$$E_s\,(avg) = \frac{wBT}{r} \frac{\int_0^{Rmax} R \int_{-\pi/3}^{\pi/3} \cos\theta\, e^{-\frac{\alpha R}{\cos\theta}}\, d\theta\, dR}{\int_0^{Rmax} R\, dR} \qquad (5)$$

Figure 3

Numerical Integration Results of

$$\int_{-\pi/3}^{\pi/3} \cos\theta \, e^{-\frac{\alpha R}{\cos\theta}} \, d\theta$$

Versus

αR

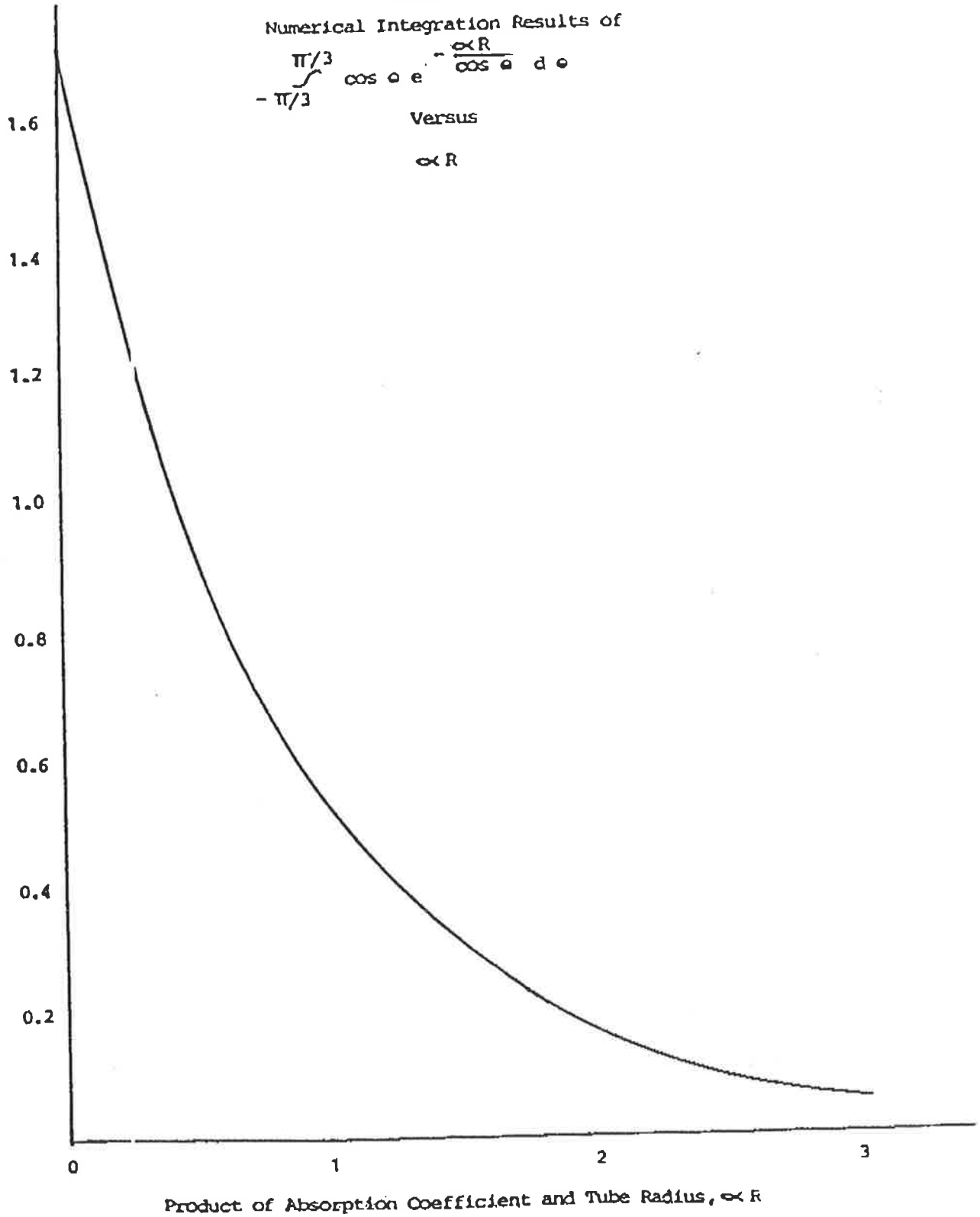
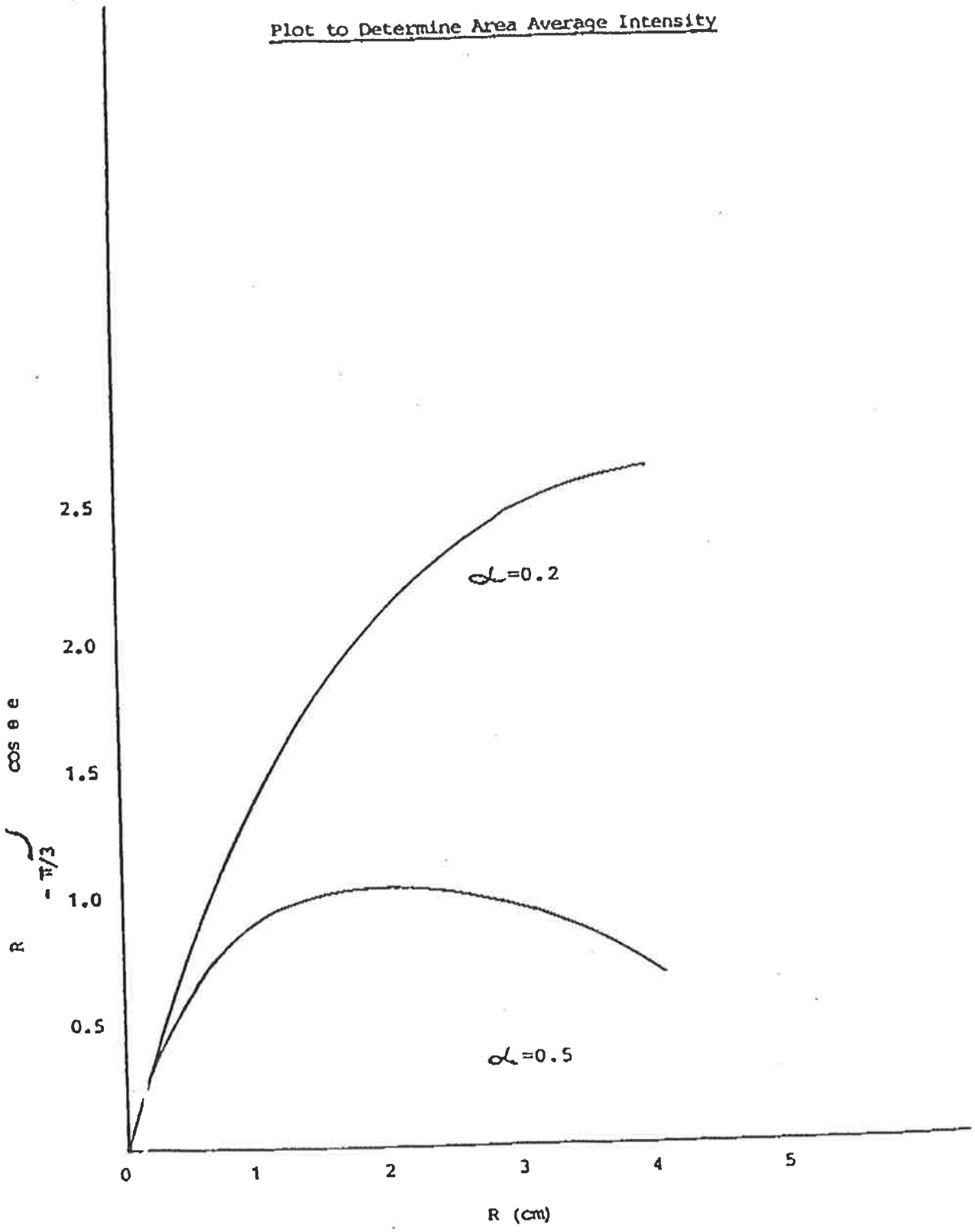


Figure 4

Plot to Determine Area Average Intensity



Equation 5 was evaluated by graphical integration for Teflon tube radii and absorption coefficients used in UTI designs (Figure 4). Source radiances were determined for two germicidal UV lamps by experimentally measuring irradiance versus distance from the lamp and averaging the calculated radiances. There was only a plus or minus two percent deviation from Lambert's cosine law in the experimental measurements up to a distance of six inches from the lamp surface. Transmittance of the Teflon was determined over several batches of extruded tubes and found to be a minimum of 80 percent.

APPENDIX D - Calculation of residence time

For the UV-LC5 disinfection unit the U-tube of advanced fluoropolymer tubing has an:

- internal diameter 11.11 mm
- axial length 636.6 mm.

The volume of water contained in the U-tube axial length is given by:

$$V = \frac{\pi D^2}{4} \times L$$

$$= 61710 \text{ mm}^3 = 0.0000617 \text{ m}^3$$

$$\Rightarrow \quad V = 0.0617 \text{ L}$$

$$\text{Residence time, } t = \frac{\text{Volume}}{\text{Flow rate (L.s}^{-1}\text{)}}$$

$$= \frac{V}{FR}$$

$$t = \frac{0.0617 \text{ L}}{FR \text{ (L.s}^{-1}\text{)}}$$

Summary Table D1

Flow rate $L.s^{-1}$	Residence time s
0.000	infinite
0.017	3.7
0.033	1.9
0.050	1.2
0.067	0.9

APPENDIX E - Microbiological data for inactivation of *Escherichia coli* and *Pseudomonas aerugicosa* in RO water by UV-LC5 disinfection unit

Number[(*)] of replicated trials	Initial number, N_0 (No.mL^{-1})	Survival number, N (No.mL^{-1})	% Survival (100 x N/N_0)	ln (N/N_0)
1	68 x 10^7	25 x 10^3	0.0037	-10.20
	86 x 10^5	18 x 10	0.0020	-10.78
2	95 x 10^6	81 x 10^2	0.0085	-9.37
	19 x 10^7	15 x 10^3	0.0078	-9.46
3	40 x 10^7	44 x 10^3	0.0110	-9.12
	52 x 10^7	44 x 10^3	0.0085	-9.37
	84 x 10^7	82 x 10^3	0.0098	-9.23
4	18 x 10^6	33 x 10^2	0.0183	-8.60
	55 x 10^7	103 x 10^3	0.0187	-8.58
5	50 x 10^6	132 x 10^2	0.0264	-8.24
	20 x 10^7	47 x 10^3	0.0234	-8.36
6	60 x 10^6	94 x 10^2	0.0157	-8.76
	52 x 10^6	112 x 10^2	0.0215	-8.45
7	55 x 10^6	118 x 10^2	0.0215	-8.45
	61 x 10^6	131 x 10^2	0.0215	-8.45
	22 x 10^6	56 x 10^2	0.025	-8.29
8	60 x 10^5	19 x 10^2	0.032	-8.05
	28 x 10^7	95 x 10^3	0.0339	-7.99
9	129 x 10^5	65 x 10^2	0.0502	-7.60
	130 x 10^7	70 x 10^4	0.0539	-7.53
10	76 x 10^5	96 x 10	0.0127	-8.97
	130 x 10^6	22 x 10^3	0.0170	-8.68
	73 x 10^5	142 x 10	0.0195	-8.54

MICROBIOLOGICAL DATA (CONTINUED)

Number(*) of replicated trials	Initial number, N_0 (No.mL^{-1})	Survival number, N (No.mL^{-1})	% Survival (100 x N/N_0)	ln (N/N_0)
11	86×10^6	23×10^3	0.0265	-8.24
	75×10^6	18×10^3	0.0243	-8.32
	11×10^6	32×10^2	0.0290	-8.15
12	91×10^6	28×10^3	0.0304	-8.10
	94×10^5	31×10^2	0.0333	-8.01
	18×10^6	57×10^2	0.0316	-8.06
13	36×10^6	15×10^3	0.0416	-7.79
	126×10^5	54×10^2	0.0425	-7.76
	32×10^6	144×10^2	0.0570	-7.70
14	69×10^6	40×10^3	0.0586	-7.44
	23×10^6	115×10^2	0.0512	-7.58
	64×10^6	39×10^3	0.0608	-7.41
15	26×10^6	101×10^2	0.0390	-7.85
	68×10^6	25×10^3	0.0360	-7.93
	46×10^6	175×10^2	0.0380	-7.88
16	19×10^6	74×10^2	0.0389	-7.85
	34×10^6	156×10^2	0.0458	-7.69
	60×10^6	23×10^3	0.0375	-7.89
17	138×10^6	84×10^3	0.0608	-7.41
	44×10^6	27×10^3	0.0602	-7.42
	36×10^6	187×10^2	0.0520	-7.56
18	34×10^6	25×10^3	0.0721	-7.24
	14×10^6	99×10^2	0.0705	-7.26
	28×10^6	20×10^3	0.0713	-7.25
19	49×10^7	103×10^2	0.0021	-10.77
	97×10^5	107	0.0011	-11.42
20	97×10^6	63×10^2	0.0064	-9.66
	130×10^6	68×10^2	0.0052	-9.86

MICROBIOLOGICAL DATA (CONTINUED)

Number(*) of replicated trials	Initial number, N_0 (No.mL^{-1})	Survival number, N (No.mL^{-1})	% Survival (100 x N/N_0)	ln (N/N_0)
21	20 x 10^7	19x 10^3	0.0095	-9.26
	63 x10^7	52 x 10^3	0.0083	-9.40
	47 x 10^7	33 x 10^3	0.0071	-9.55
22	41 x 10^6	60 x 10^2	0.0145	-8.84
	140 x 10^6	22x 10^3	0.0159	-8.75
23	30 x 10^7	64 x 10^3	0.0212	-8.46
	90 x 10^6	21 x 10^3	0.0233	-8.37
24	59 x 10^6	81 x 10^2	0.0137	-8.90
	106 x 10^6	100 x 10^2	0.0094	-9.27
25	60 x 10^6	85 x 10^2	0.0142	-8.86
	45 x 10^6	56 x 10^2	0.0124	-9.00
	100 x 10^6	136 x 10^3	0.0136	-8.90
26	120 x 10^5	26 x 10^2	0.0216	-8.44
	25 x 10^7	57 x 10^3	0.0228	-8.37
27	17 x 10^6	65 x 10^2	0.0384	-7.87
	120 x 10^7	48 x 10^4	0.0401	-7.82
28	90 x 10^5	90 x 10	0.0100	-9.21
	22 x 10^6	25 x 10^2	0.0112	-9.11
	95 x 10^5	14 x 10^2	0.0146	-8.83
29	94 x 10^6	165 x 10^2	0.0175	-8.65
	65x 10^6	103 x 10^3	0.0187	-8.58
	60 x 10^5	114x 10	0.0190	-8.57
30	85 x 10^5	25 x 10^2	0.0293	-8.24
	172 x 10^5	46 x 10^2	0.0264	-8.14
	77 x 10^6	191 x 10^2	0.0248	-8.30
31	35 x 10^6	119 x 10^2	0.0341	-7.98
	33 x 10^6	130 x 10^2	0.0401	-7.82
	185 x 10^5	68 x 10^2	0.0369	-7.90

MICROBIOLOGICAL DATA (CONTINUED)

Number(*) of replicated trials	Initial number, N_0 (No.mL^{-1})	Survival number, N (No.mL^{-1})	% Survival (100 x N/N_0)	ln (N/N_0)
32	78×10^6	38×10^3	0.0485	-7.63
	25×10^6	111×10^2	0.0452	-7.70
	52×10^7	27×10^3	0.0512	-7.58
33	34×10^6	99×10^2	0.0290	-8.15
	102×10^6	25×10^3	0.0242	-8.33
	43×10^6	93×10^2	0.0217	-8.44
34	16×10^6	44×10^2	0.0275	-8.20
	38×10^6	91×10^2	0.0240	-8.33
	56×10^6	158×10^2	0.0282	-8.17
35	50×10^6	24×10^3	0.0475	-7.65
	42×10^6	166×10^2	0.0395	-7.84
	28×10^6	117×10^2	0.0419	-7.78
36	44×10^6	27×10^3	0.0601	-7.42
	180×10^5	107×10^2	0.0594	-7.43
	36×10^6	20×10^3	0.0558	-7.49
37	30×10^7	39×10^2	0.0013	-11.25
	55×10^6	49 x 10	0.0009	-11.62
38	95×10^6	30×10^2	0.0032	-10.35
	140×10^6	62×10^2	0.0043	-10.05
39	100×10^6	84×10^2	0.0084	-9.39
	66×10^7	46×10^3	0.0069	-9.58
	39×10^7	16×10^3	0.0041	-10.10
40	27×10^6	34×10^2	0.0124	-8.99
	130×10^6	172×10^2	0.0132	-8.93
41	160×10^6	34×10^3	0.0213	-8.45
	70×10^6	128×10^2	0.0182	-8.61
42	96×10^6	56×10^2	0.0058	-9.76
	70×10^6	66×10^2	0.0094	-9.27

MICROBIOLOGICAL DATA (CONTINUED)

Number(*) of replicated trials	Initial number, N_0 ($No.mL^{-1}$)	Survival number, N ($No.mL^{-1}$)	% Survival ($100 \times N/N_0$)	ln (N/N_0)
43	75×10^6	65×10^2	0.0086	-9.36
	34×10^6	22×10^2	0.0064	-9.66
	110×10^7	99×10^3	0.0090	-9.32
44	80×10^5	103×10	0.0129	-8.96
	25×10^7	30×10^3	0.0119	-9.04
45	108×10^5	24×10^2	0.0224	-8.40
	60×10^7	128×10^3	0.0214	-8.45
46	101×10^5	57×10	0.0056	-9.79
	26×10^6	19×10^2	0.0073	-9.53
	91×10^6	56×10	0.0061	-9.70
47	135×10^6	20×10^3	0.0151	-8.80
	110×10^5	19×10^2	0.0169	-8.69
	77×10^6	108×10^2	0.0140	-8.87
48	105×10^5	23×10^2	0.0215	-8.45
	19×10^6	39×10^2	0.0208	-8.48
	60×10^6	134×10^2	0.0224	-8.40
49	34×10^6	80×10^2	0.0236	-8.35
	128×10^5	36×10^2	0.0281	-8.18
	80×10^6	24×10^3	0.0295	-8.13
50	81×10^6	29×10^3	0.0356	-7.94
	28×10^6	113×10^3	0.0402	-7.82
	72×10^6	29×10^3	0.0398	-7.83
51	44×10^6	67×10^2	0.0151	-8.80
	25×10^7	29×10^2	0.0115	-9.07
	38×10^6	42×10^2	0.0110	-9.12
52	160×10^5	33×10^2	0.0204	-8.50
	18×10^6	40×10^2	0.0219	-8.43
	42×10^6	93×10^2	0.0220	-8.17

MICROBIOLOGICAL DATA (CONTINUED)

Number[(*)] of replicated trials	Initial number, N_0 (No.mL^{-1})	Survival number, N (No.mL^{-1})	% Survival (100 x N/N_0)	ln (N/N_0)
53	24×10^6	77×10^2	0.0321	-8.04
	36×10^6	114×10^2	0.0316	-8.06
	38×10^6	109×10^2	0.0286	-8.16
54	56×10^6	26×10^3	0.0458	-7.69
	26×10^6	108×10^2	0.0416	-7.79
	52×10^6	20×10^3	0.0386	-7.86
55	28×10^7	31×10^2	0.0011	-11.42
	35×10^6	31 x 10	0.0009	-11.62
56	95×10^6	23×10^2	0.0024	-10.64
	100×10^6	24×10^2	0.0024	-10.64
57	20×10^7	70×10^2	0.0035	-10.26
	83×10^7	26×10^3	0.0031	-10.38
58	36×10^6	19×10^2	0.0053	-9.85
	25×10^7	120×10^2	0.0048	-9.94
59	24×10^7	31×10^3	0.0129	-8.96
	120×10^6	130×10^2	0.0108	-9.13
60	140×10^6	45×10^2	0.0032	-10.35
	47×10^6	23×10^2	0.0048	-9.94
61	112×10^6	64×10^2	0.0057	-9.77
	83×10^6	33×10^2	0.0040	-10.13
62	80×10^7	83×10^3	0.0103	-9.18
	100×10^5	102 x 10	0.0102	-9.19
63	148×10^5	24×10^2	0.0158	-8.57
	93×10^7	153×10^3	0.0164	-8.72
64	85×10^5	19 x 10	0.0022	-10.73
	60×10^6	78 x 10	0.0013	-11.25
	147×10^5	30 x 10	0.0020	-10.82

MICROBIOLOGICAL DATA (CONTINUED)

Number(*) of replicated trials	Initial number, N_0 (No.mL^{-1})	Survival number, N (No.mL^{-1})	% Survival (100 x N/N_0)	ln (N/N_0)
65	61×10^6	22×10^2	0.0035	-10.26
	74×10^6	35×10^2	0.0047	-9.97
	90×10^5	50×10	0.0055	-9.81
66	77×10^5	45×10	0.0058	-9.76
	25×10^6	18×10^2	0.0070	-9.57
	84×10^6	68×10^2	0.0081	-9.42
67	39×10^6	37×10^2	0.0095	-9.26
	17×10^6	15×10^2	0.0084	-9.39
	34×10^6	39×10^2	0.0116	-9.06
68	81×10^6	101×10^2	0.0125	-8.99
	172×10^5	27×10^2	0.0157	-8.76
	40×10^6	53×10^2	0.0132	-8.93
69	42×10^6	21×10^2	0.0050	-9.90
	24×10^6	20×10^2	0.0085	-9.37
	22×10^7	18×10^3	0.0082	-9.41
70	14×10^6	17×10^2	0.0120	-9.03
	18×10^6	31×10^2	0.0172	-8.67
	42×10^6	65×10^2	0.0153	-9.79
71	24×10^6	43×10^2	0.0179	-8.63
	60×10^6	91×10^2	0.0151	-8.80
	40×10^6	66×10^2	0.0165	-8.71
72	58×10^6	16×10^3	0.0275	-8.20
	32×10^6	96×10^2	0.0298	-8.12
	76×10^6	23×10^3	0.0304	-8.10

(*) - The replicated trials were carried out at the same experimental conditions (concentration of absorbing agent or SS, pH, UV transmittance ...) with those disinfection trials of Table 4.1.

APPENDIX F - Reynold number calculation

Reynolds found that variables affecting transition from laminar to turbulent flow in a pipe were: pipe diameter (D); fluid (liquid) velocity (υ); fluid density (ρ) and fluid viscosity (μ). These can be arranged in a single dimensionless group calls the Reynolds number:

$$Re = \frac{D\upsilon\rho}{\mu}$$

For pipe flow:

$Re < 2{,}1000$	- laminar flow
$2{,}100 < Re < 10{,}000$	- transition region
$Re > 10{,}000$	- turbulent flow

For the water at 25°C:

$\rho = 1000\ kg\ m^{-3}$
$\mu = 1.0\ cP = 1 \times 10^{-3}$ (Pa. s)

For the LC5- disinfection unit flow- tubing:

internal diameter (D) = 11.11 mm = 0.0011 m
axial length (L) = 636.6 mm = 0.6366 m

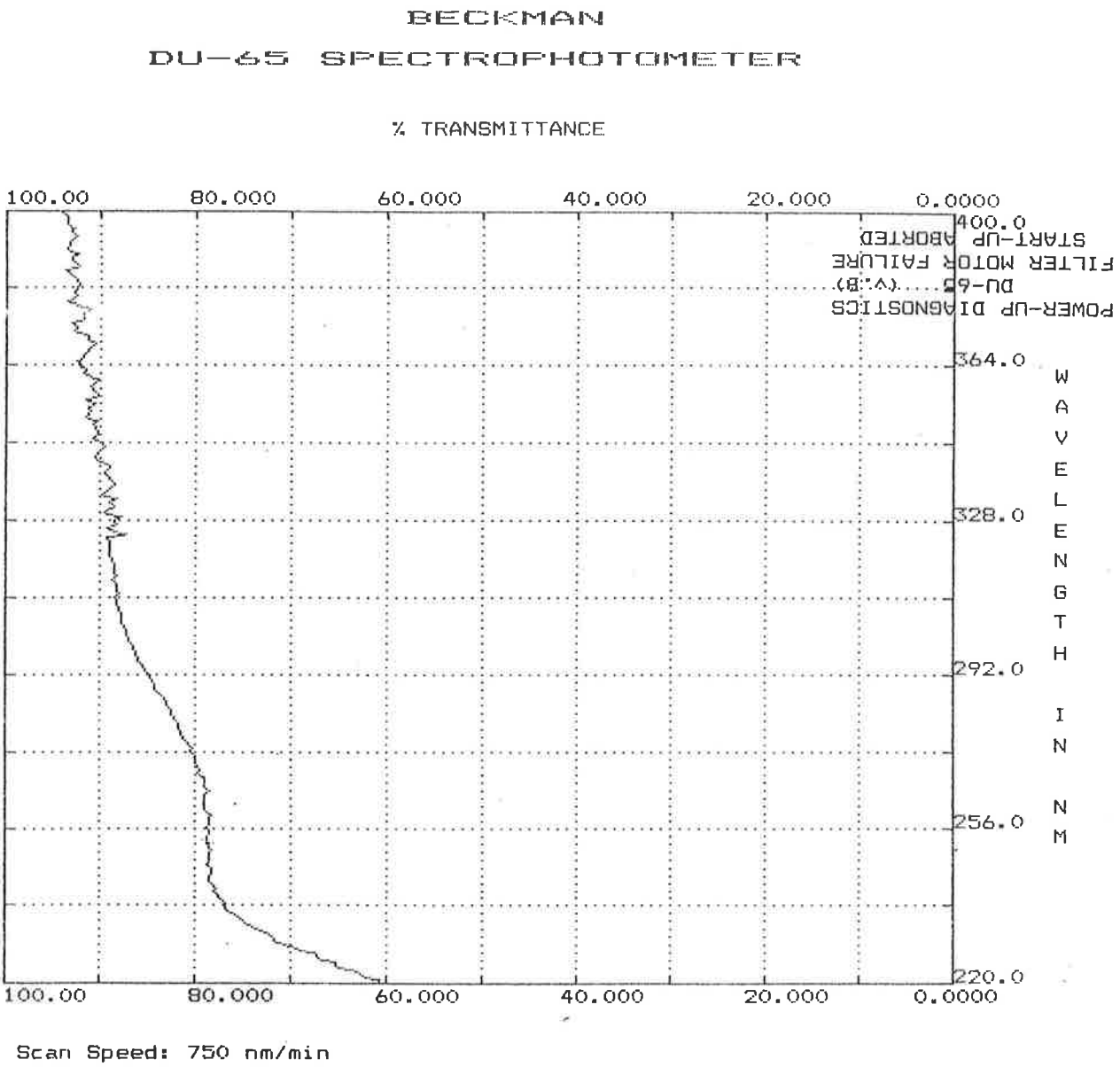
Hence:

$$Re = \frac{0.0011 \times \upsilon \times 1000}{1 \times 10^{-3}}$$

Appendix F Summary Table

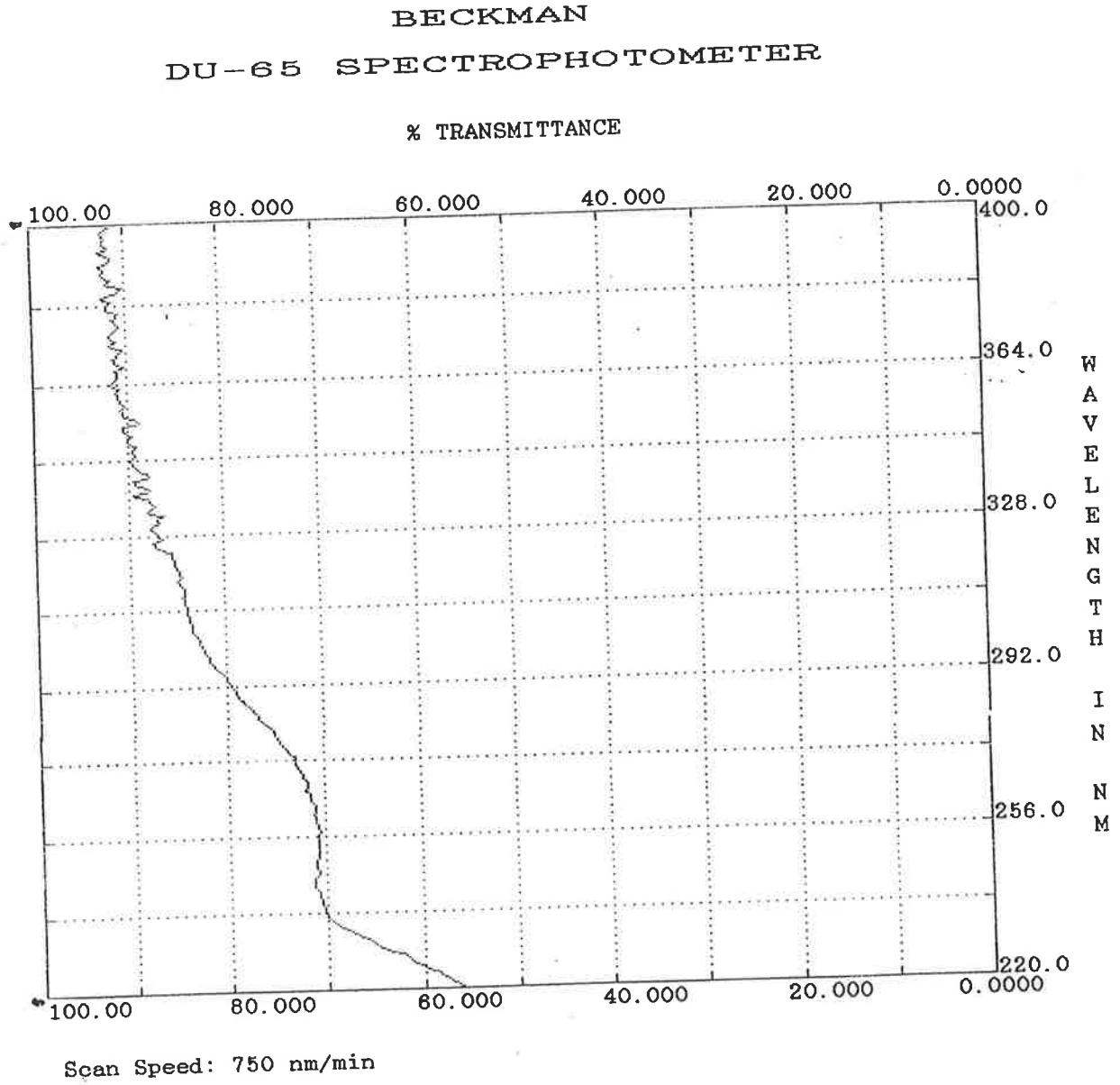
Flow rate ($L.s^{-1}$)	$\upsilon(m.s^{-1})$ x 10^3	Reynold number
0.000	0	0
0.017	18	1.98 E7
0.033	35	3.85 E7
0.050	53	5.83 E7
0.067	71	7.81 E7

APPENDIX G - Ultraviolet - visible spectra of test micro-organisms with or without the presence of absorbing agent (coffee) or suspended solids (Celite 503)



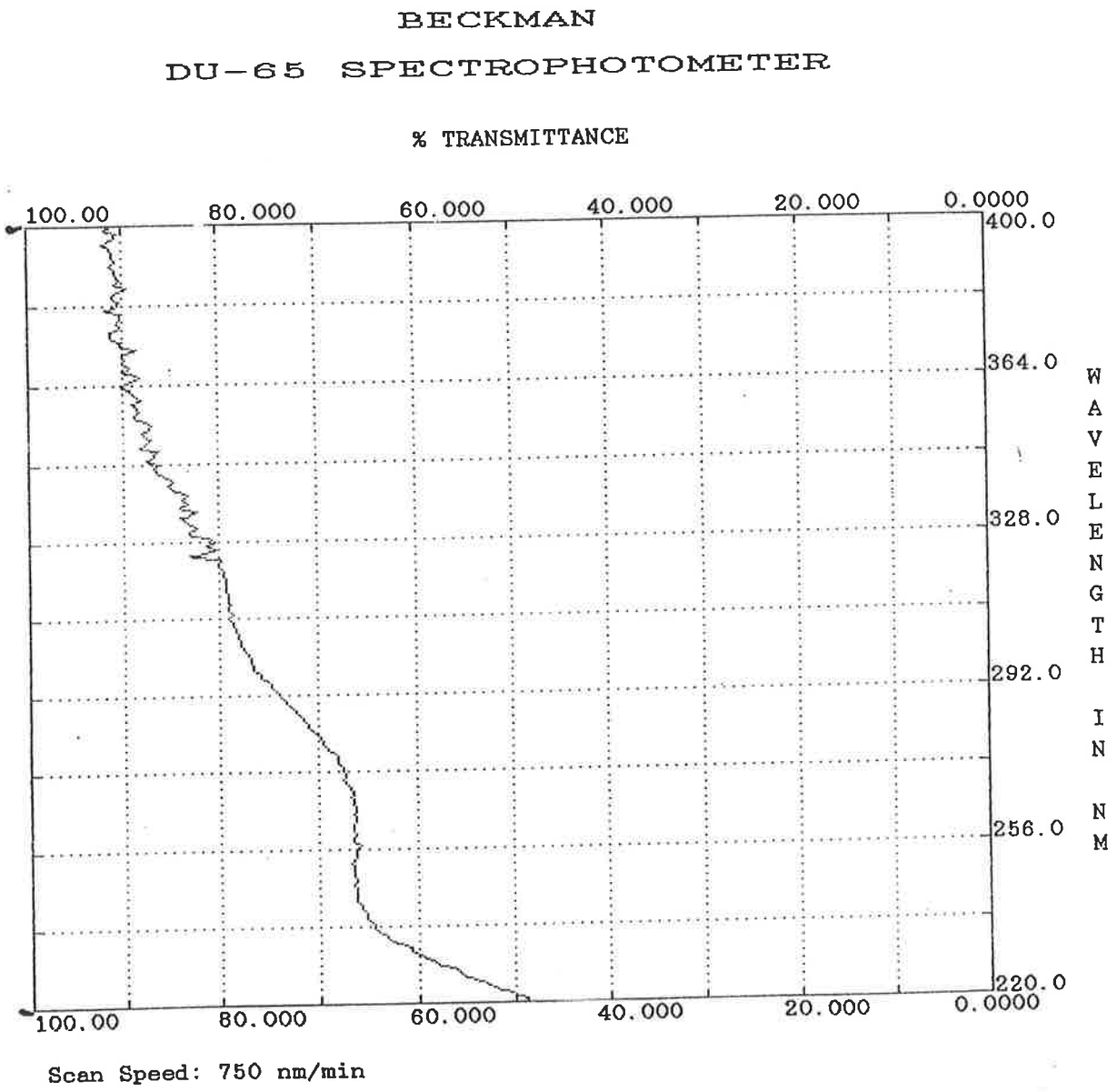
UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1})

28 July 98

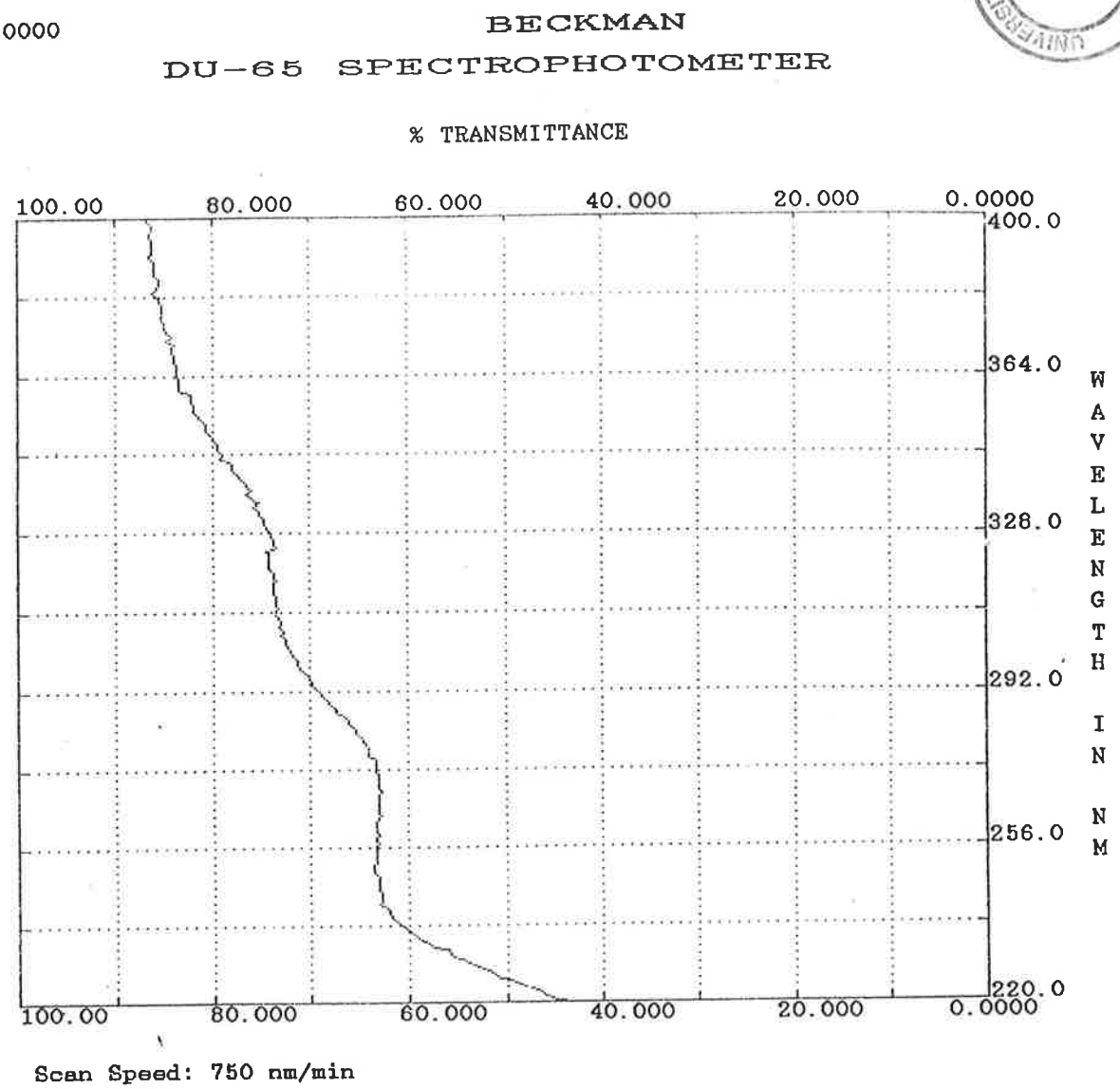


UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of absorbing agent (coffee) 0.001 g.L^{-1} .

28 July 98

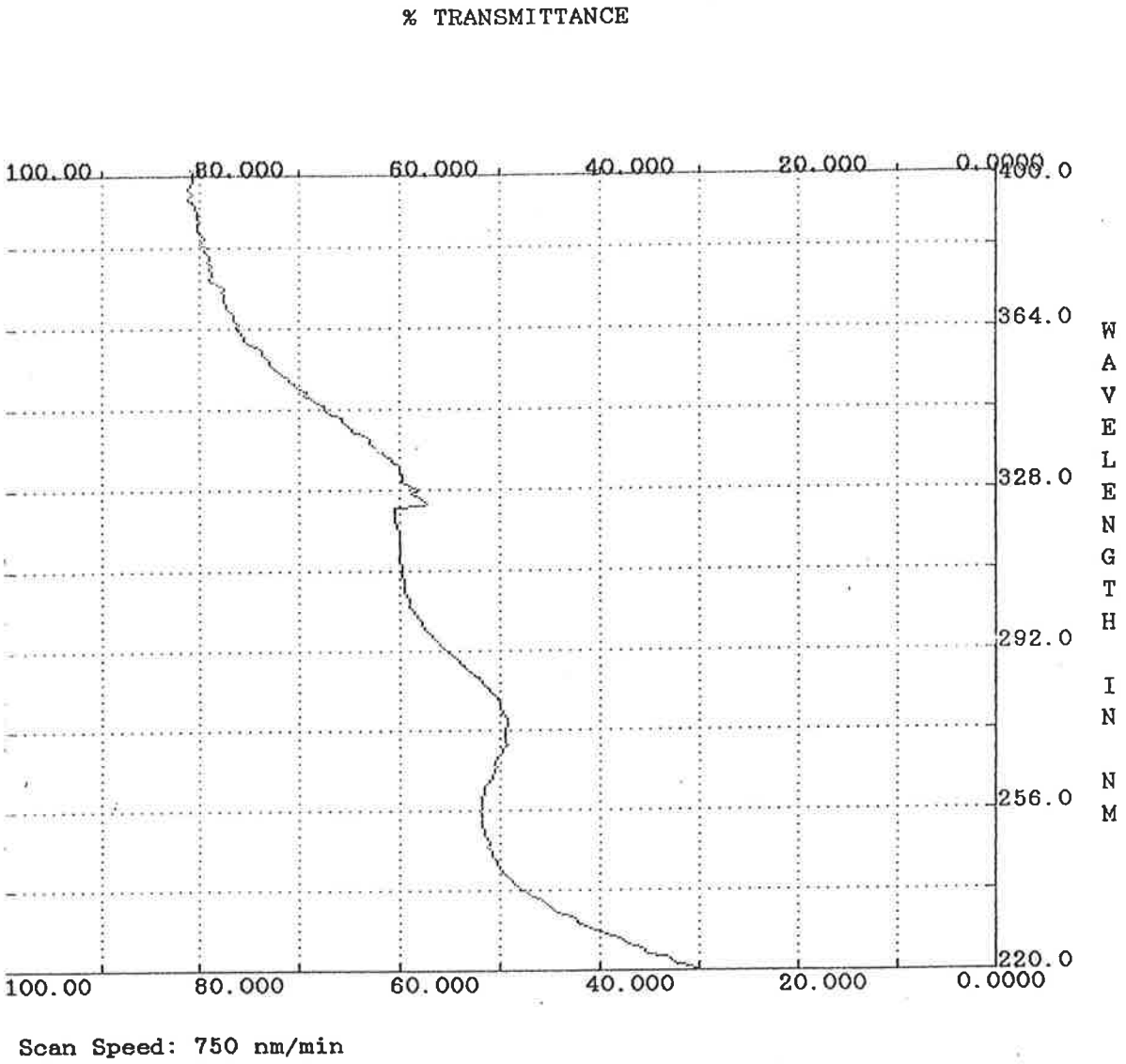


UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of absorbing agent (coffee) 0.005 g.L^{-1}



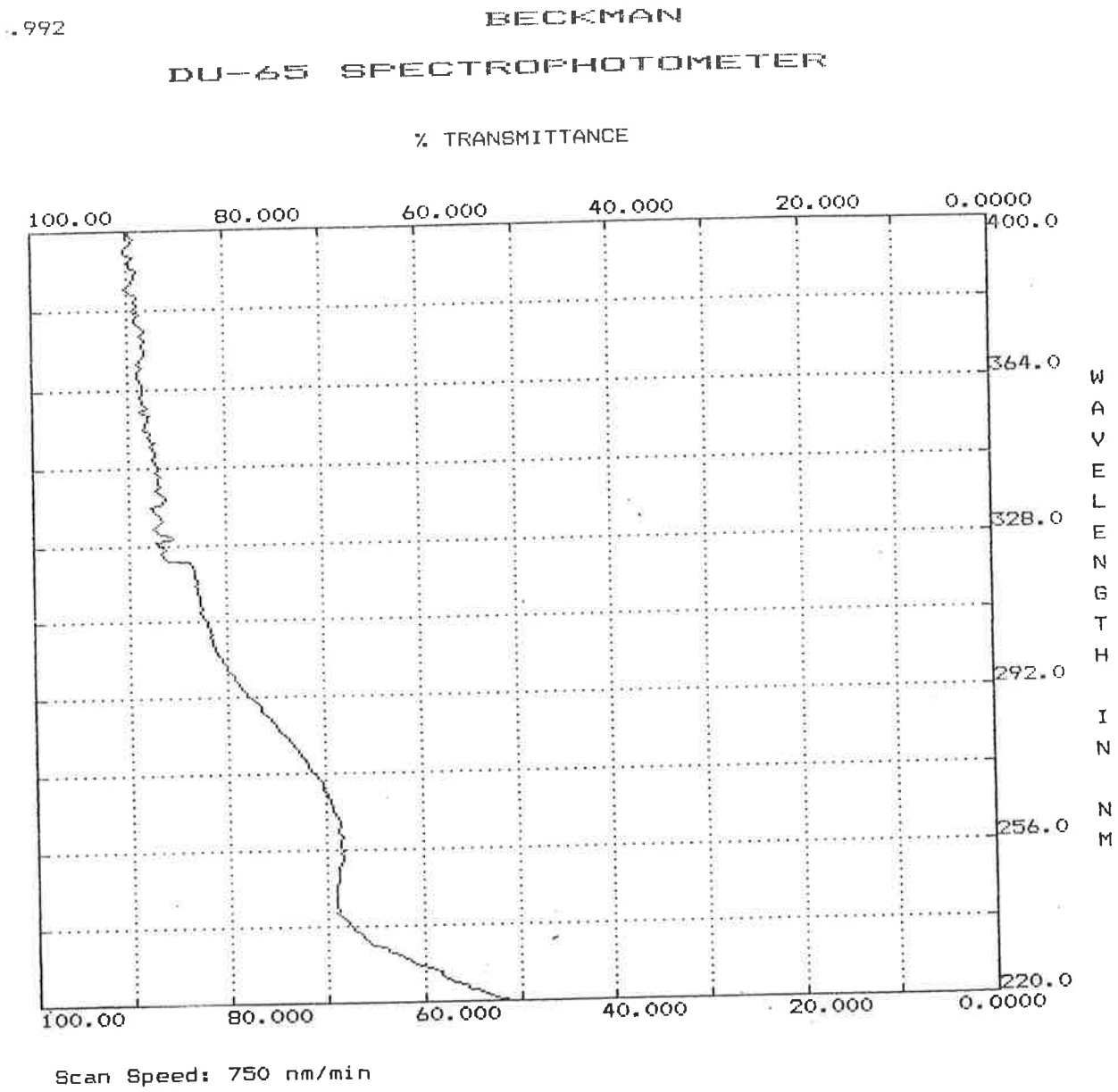
UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of absorbing agent (coffee) 0.01 g.L^{-1}

3/17



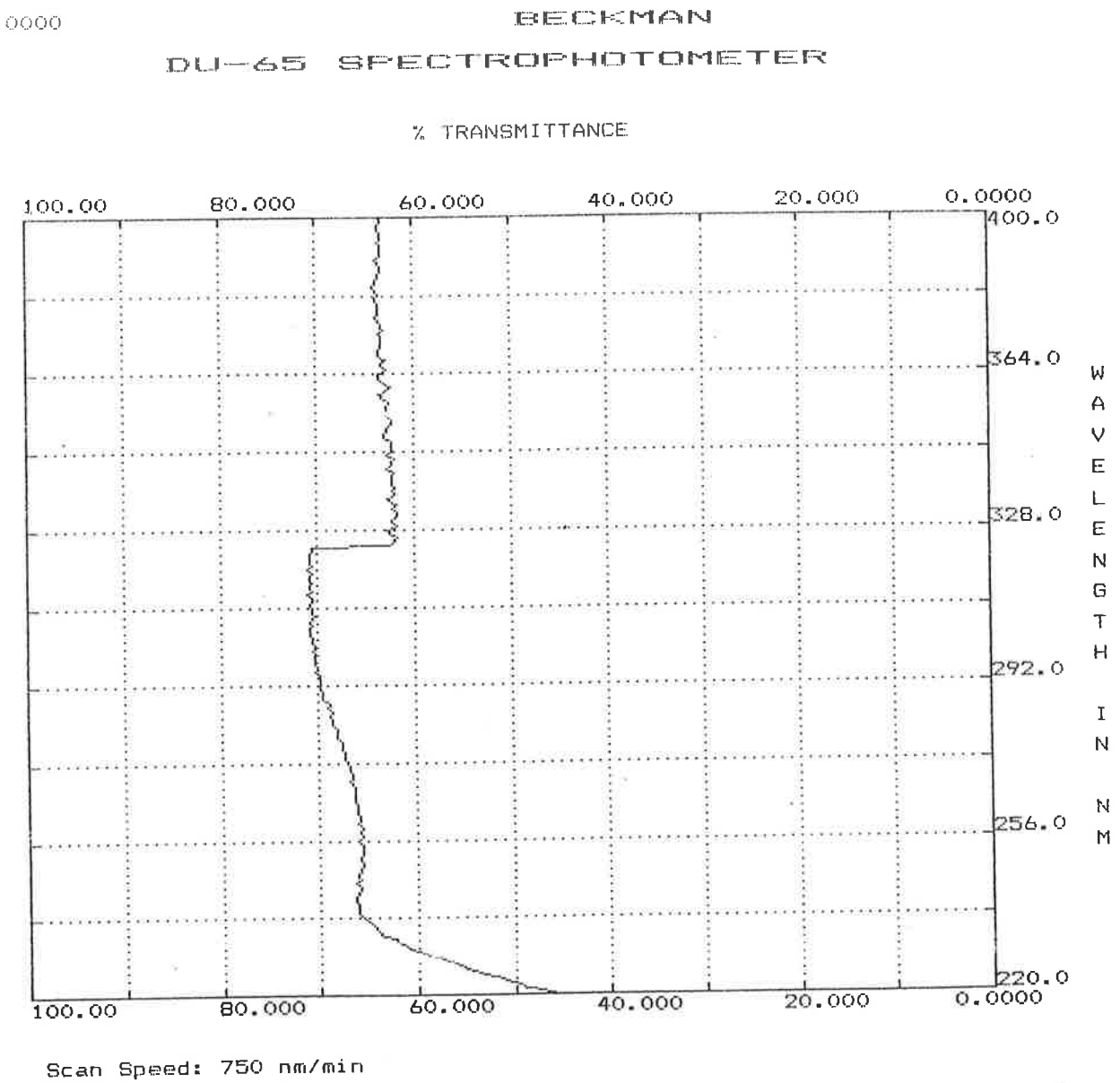
UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of absorbing agent (coffee) 0.03 g.L^{-1}

6 Aug 98



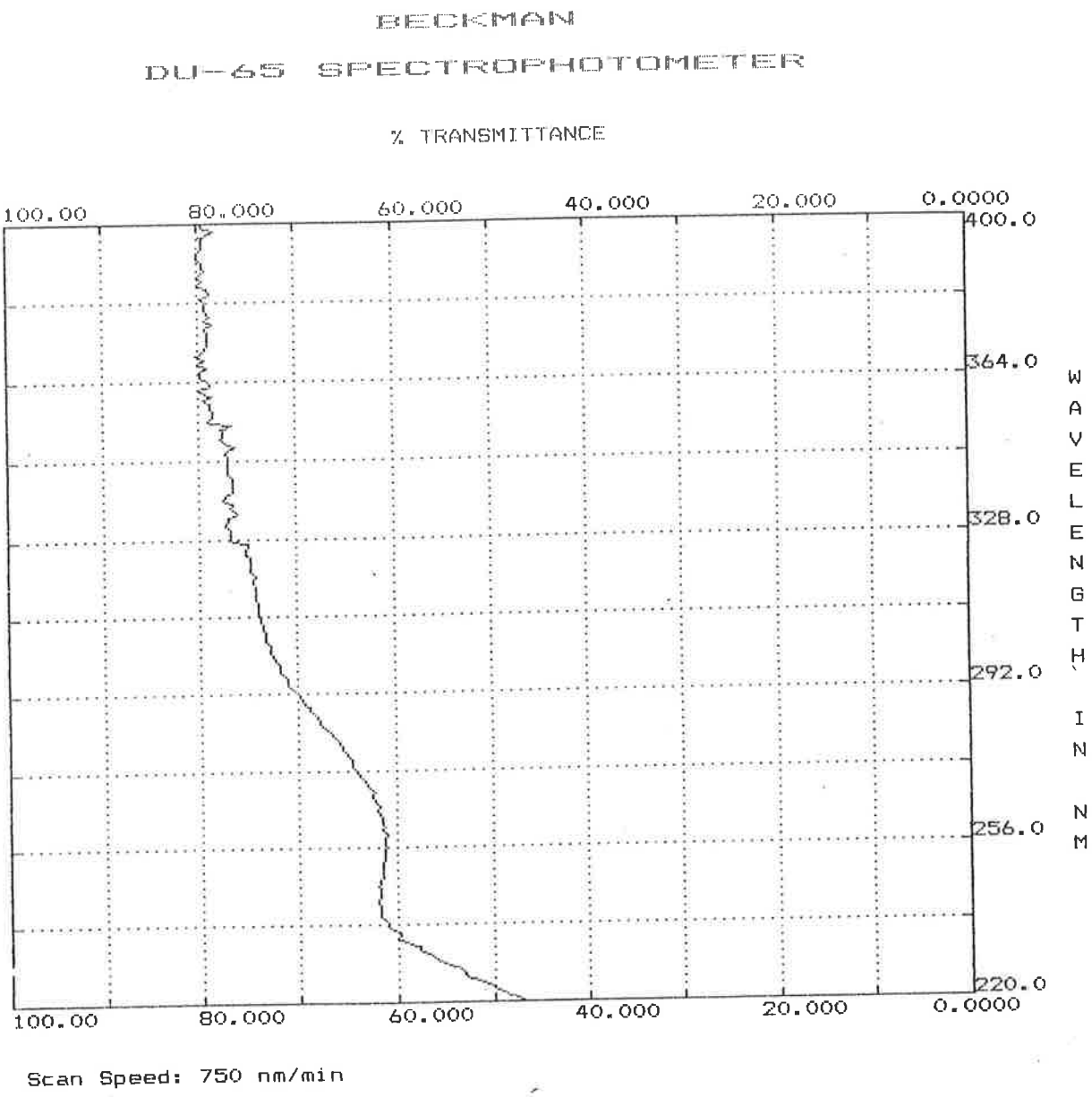
UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of suspended solid (celite 503) 0.01 g.L^{-1}

4 August

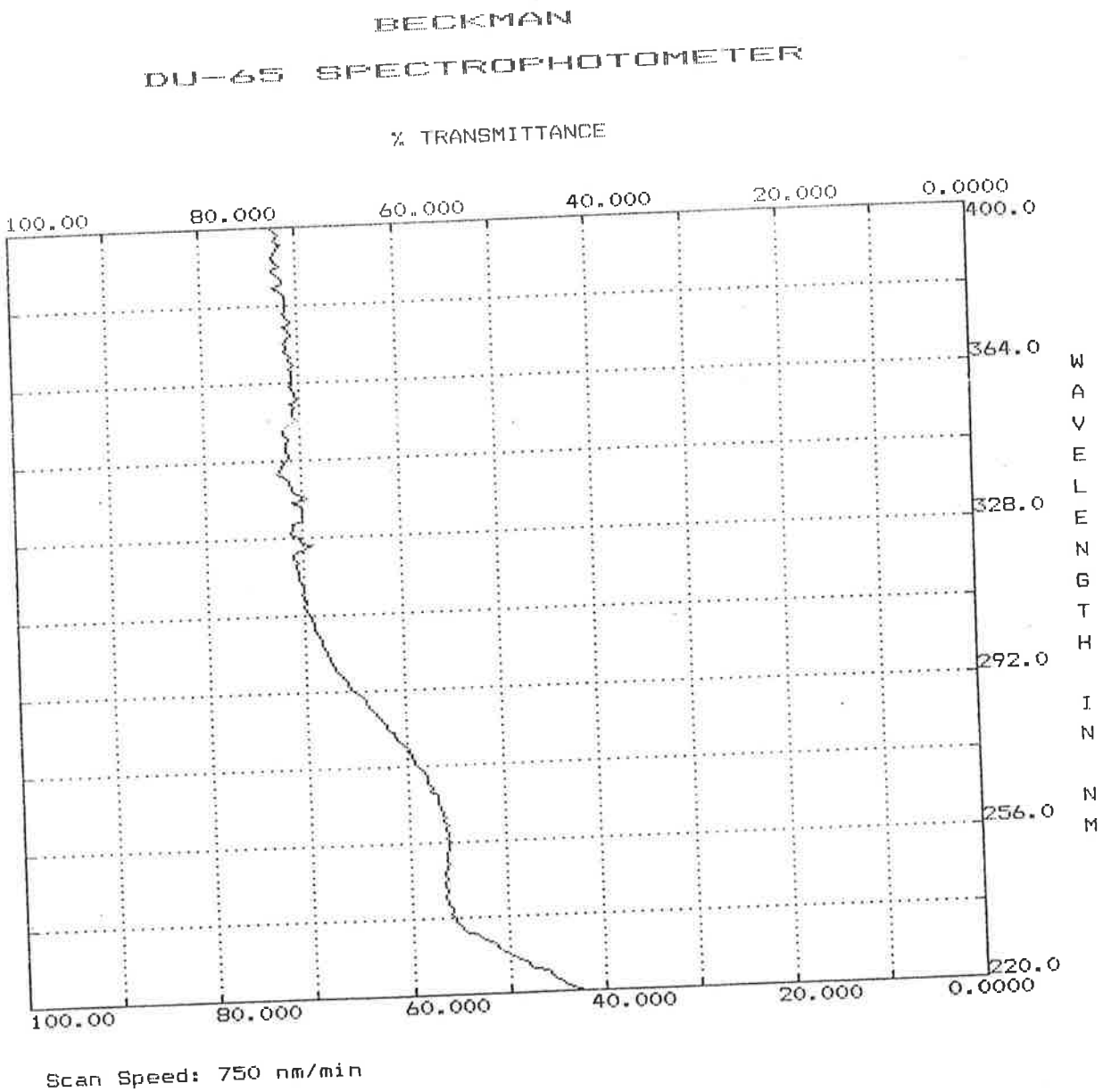


UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of suspended solid (celite 503) 0.05 g.L^{-1}

6 Aug.



UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of suspended solid (celite 503) 0.1 g.L^{-1}

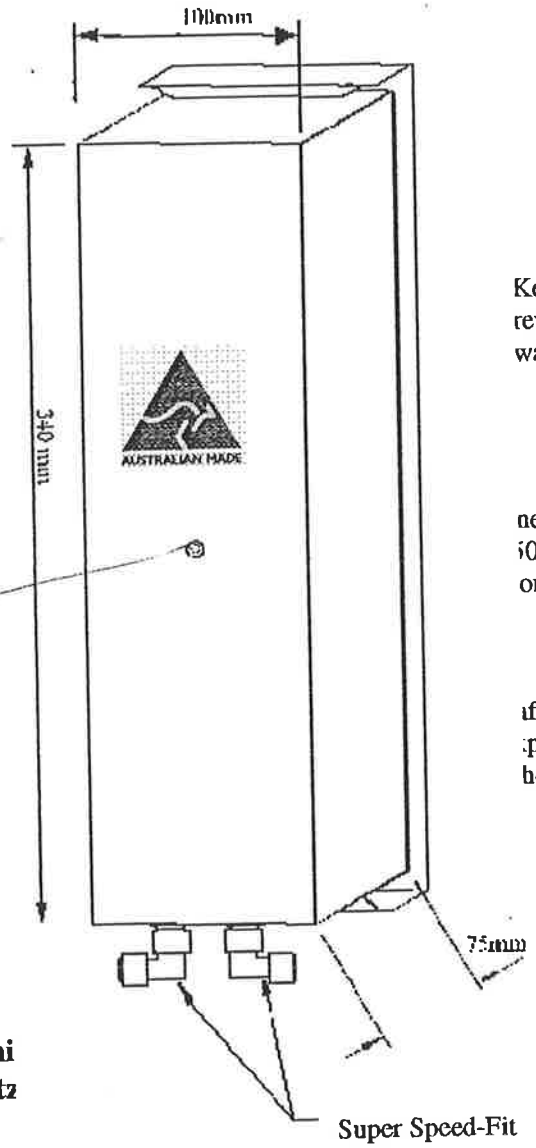


UV spectra of *Pseudomonas aeruginosa* (~ 10^7 cells.mL^{-1}) with the presence of suspended solid (celite 503) 0.3 g.L^{-1}

APPENDIX H - Photographs of the UV-LC 5 disinfection unit

LC 5

Ultraviolet Disinfection Unit



Disinfects water at a rate of up to 5 L/min

Unit enclosed in robust powder coated aluminium

Glowing jewel type indicator shows operation

Advanced fluoropolymer tube carries water along side of U.V. mercury vapour lamp

Keyhole mounting on reverse of casing for easy wall mounting

ne 20 watt lamp to provide 500 hrs of running time. 12 onths of continual use

ıfety switch to prevent posure to U.V. radiation hen unit is opened.

Super Speed-Fit push-in fittings.

***No possible contami with shattered quartz**

***No possible contamination with mercury**

PROUDLY AUSTRALIAN MANUFACTURED BY

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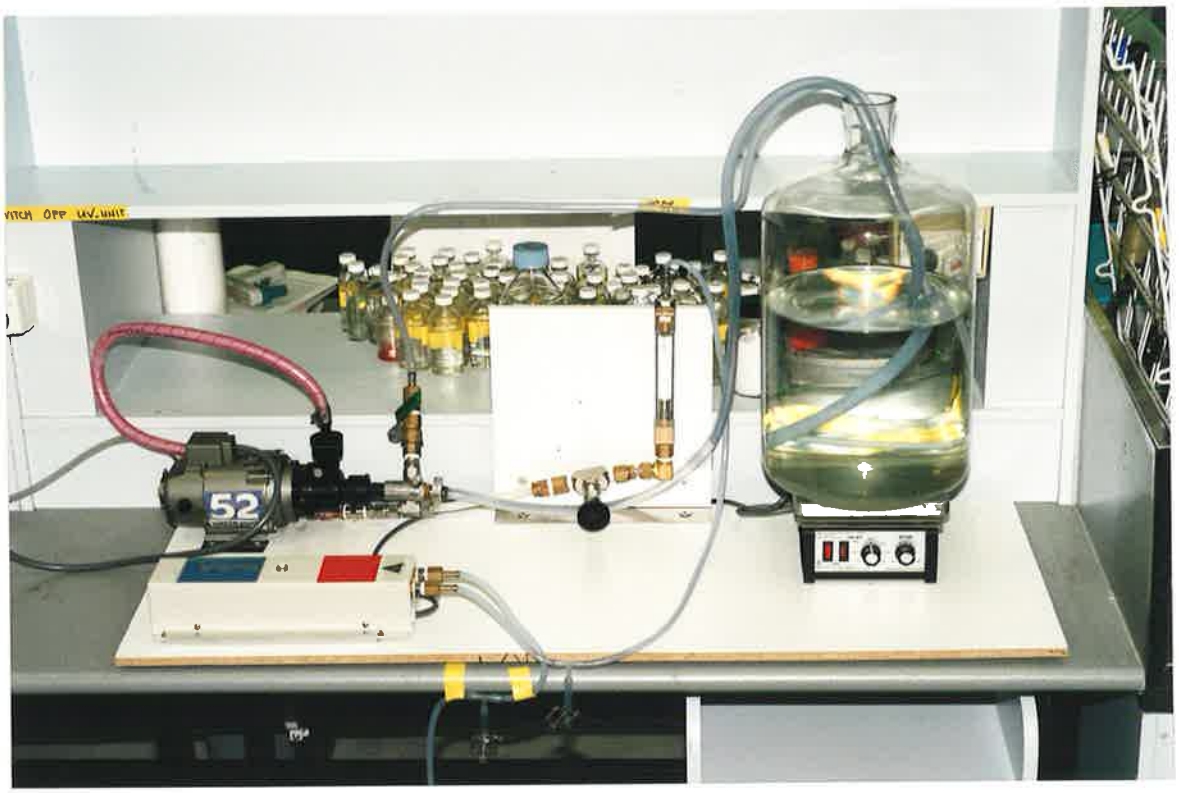

Experimental set up

APPENDIX I - Test for absorption of bacterial cells on Celite 503 and possible biocidal effects of coffee and Celite 503 on test micro-organisms

a- Test for absorption of bacteria cells on Celite 503

test micro-organism	initial number of viable cells (mL^{-1})				
	control sample	$3g.L^{-1}$ Celite 503			
	1-fold supernatant	1-fold supernatant	2-fold supernatant	3-fold supernatant	4-fold supernatant
E. coli	$58x10^6$	$36x10^6$	$85x10^5$	$50x10^4$	$68x10^3$
	$76x10^6$	$60x10^6$	$58x10^5$	$69x10^4$	$41x10^3$
	$22x10^6$	$28x10^6$	$15x10^5$	$25x10^4$	$33x10^3$
P. aeruginosa	$180x10^6$	$204x10^6$	$152x10^5$	$147x10^4$	$187x10^3$
	$192x10^6$	$225x10^6$	$148x10^5$	$196x10^4$	$208x10^3$
	$38x10^6$	$51x10^6$	$23x10^5$	$35x10^4$	$58x10^3$

b- Test for biocidal effect of coffee and Celite 503 on test micro-organisms

test micro-organism	initial number of viable cells (mL^{-1})			
	control sample	$0.03g.L^{-1}$ coffee	control sample	$0.03g.L^{-1}$ Celite 503
E. coli	$51x10^6$	$66x10^5$	$35x10^6$	$25x10^6$
	$71x10^6$	$57x10^6$	$31x10^7$	$55x10^7$
	$208x10^5$	$172x10^5$	$70x10^7$	$55x10^7$
P. aeruginosa	$60x10^6$	$90x10^6$	$34x10^6$	$50x10^6$
	$174x10^5$	$185x10^5$	$50x10^6$	$40x10^6$
	$94x10^6$	$78x10^6$	$36x10^6$	$52x10^6$

APPENDIX J - Refereed publications from this investigation

Ha Thi Nguyen, K. R. Davey, T. Gardner aand C. J. Thomas. 1998. Effect of transmittance and suspended solids on the efficacy of UV disinfection of water. Australasian Chemical Engineering Conference, CHEMECA'98 *Creating Competitive Resources*, Port Douglas, North Queensland, 28-30 September, 5pp.

NOTATION

a, b, c, m	Empirical coefficients.	
AA	Absorbing agent	
C	Concentration of solution	$mol.L^{-1}$
D	UV dose	$\mu W.s.cm^{-2}$
FR	Volumetric flow rate	$L.s^{-1}$
I	Intensity of UV radiation	$\mu W.cm^{-2}$
I_0	Intensity of the incident light	$watt.cm^{-2}$
k	Inactivation rate constant	$(\mu W.s)^{-1}.cm^2$
l	Path length of the absorbing solution	cm
L	Axial length of U advanced fluoropolymer tube	mm
m	An intensity-average function	
n	The threshold number of damage sites required for inactivation	
N_o	The initial number of bacteria present	$number.ml^{-1}$
N_t	The number of bacteria surviving at time t	$number.ml^{-1}$
N_{pr}	The number of bacteria after repair	$number.ml^{-1}$
p,q	Constant	
RR	Bacterial repair rate	
R^2	Correlation coefficient	
S	Output of UV energy	watt
SS	Suspended solids	
t	Mean residence time	s
T	UV transmittance	%
u	Velocity of waste-water through the reactor	$cm.s^{-1}$
V	Volume	L
x	Distance travelled by waste-water while exposed to UV light	cm

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