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An Investigation into the Optimisation of a Solar Photocatalytic Continuous Flow Disinfection System

David Misstear

A thesis submitted for the degree of Doctor of Philosophy to the University of Dublin, Trinity College.

June, 2010

The research for this thesis was conducted at the Department of Civil, Structural and Environmental Engineering, University of Dublin, Trinity College, Ireland.



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DECLARATION

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SUMMARY

Solar photocatalytic disinfection is thought to be a safe, effective, low-cost method of drinking water treatment and would be applicable, for example, to village-scale systems in developing countries. In this thesis, an investigation is carried out into factors affecting the photocatalytic disinfection of water.

The photolytic and photocatalytic inactivation of *E. coli* and subsequent post-irradiation events were studied under both low-intensity artificial UV light and sunlight in a small-scale compound parabolic collector (CPC). From preliminary experiments, a suitable concentration of 50 mg/L titanium dioxide (TiO₂) was chosen for the photocatalytic experiments. The addition of TiO₂ greatly increased the inactivation rate of bacteria under both light sources. No regrowth was observed following any of the experiments. However, in the experiments where no catalyst was used, there were still culturable cells 48 hours after sampling for samples taken at 60 minutes under the artificial UV light and at 180 minutes under natural sunlight. When photocatalyst was introduced, no viable cells were detected 48 hours after sampling for the 15 minute and subsequent samples either under the UV light or under natural sunlight.

The use of sterile or distilled water can give results which may be misleading when applied to practical applications. It is reported that in distilled water, in the absence of certain ions, bacterial cell walls are weakened by the loss of magnesium and calcium ions. Therefore post-irradiation experiments were conducted in a full-scale CPC under natural sunlight using distilled water, rainwater and river water. When river water was used in the absence of catalyst, regrowth of *E. coli* was witnessed post-irradiation, despite the *E. coli* concentration reaching the Method Detection Limit (MDL) during the illumination period. When catalyst was introduced, the number of bacteria continued to decline in the dark post-treatment.

An investigation into the effect of different light/dark periods on the photolytic and photocatalytic disinfection of *E. coli* was undertaken at laboratory scale under UV light. For photolysis, it was seen that the number of dark and light periods has very little effect on disinfection, once the illuminated area is kept constant. In photocatalytic tests, it was found that increasing the number of light periods, while keeping the illuminated area constant, had a

beneficial effect on disinfection, both in terms of time to Method Detection Limit (MDL) and Effective Disinfection Time (EDT).

The photolytic and photocatalytic disinfection of three bacteriophages (MS2, Φ X174, PR772) under both artificial UV irradiation and natural sunlight was studied. A large variation was seen in the relative responses of phages to the two light sources, while solar photocatalysis showed promising disinfection capabilities for all three phages.

Finally, the design of a suitable fixed photocatalytic support was researched, using computational fluid dynamics (CFD) to simulate the percentage of tracked particles coming into contact with the irradiated surface. It was found that a conical frustrum design was more than three times as efficient as a similarly-sized conventional cylindrical insert with regard to this parameter, while arrangements of shorter frusta exhibited even more promising traits.

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LIST OF ABBREVIATIONS

•OH	hydroxyl radical
AFM	atomic force microscopy
AMP	adenosine monophosphate
AMT	Advanced Microscopy Techniques
AOP	advanced oxidation process
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BDMA	benzyl dimethylamine
CAT	chloramphenicol acetyl transferase
CFD	computational fluid dynamics
CFU	colony forming unit(s)
CMBR	completely mixed batch reactor
CPC	compound parabolic collector
DBP	disinfection byproduct
DDSA	dodecenyl succinic anhydride
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DPM	discrete phase modelling
EDT	effective disinfection time
MDA	malondialdehyde
MDL	method detection limit
MDR	mutagenic DNA repair
MNA	methyl nadic anhydride
NER	nucleotide excision repair
NTU	nephelometric turbidity unit
PET	polyethylene terephthalate
PFU	plaque forming unit(s)
PTR	parabolic trough reactor
PVA	polyvinyl alcohol
RNA	ribonucleic acid
ROS	reactive oxygen species
SEM	scanning electron microscope
SOD	superoxide dismutase
SODIS	solar disinfection
SPC-DIS	solar photocatalytic disinfection
TEM	transmission electron microscopy
THM	tri-halo methane
TiO ₂	titanium dioxide
UN	United Nations
UV	ultraviolet
VOC	volatile organic compound
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background

It is estimated that water-related diseases claim the lives of roughly 3.6 million people every year, with the vast majority of those cases occurring in developing countries (WHO, 2008). In its Millennium Development Goals, the United Nations set a target to halve, by 2015, the number of people without access to basic sanitation and a supply of clean drinking water (UN, 2000). Providing microbiologically clean water to communities in developing countries is one of the most important and challenging jobs facing engineers today. The process of solar photocatalysis could provide a cheap and effective solution to this problem, as the disinfection stage of drinking water treatment is currently energy and/or resource intensive. The standard method is chlorination, but this yields problems in terms of taste and the potential for the creation of toxic compounds, such as tri-halo methanes (THMs). Other options such as ozonation are used, but this has a particularly high energy requirement. Artificial UV light created by low or medium pressure mercury vapour lamps has been proved to be effective and is also commonly used but has relatively high energy and maintenance requirements (Twort *et al.*, 2000).

Solar disinfection has been proven to be effective against a number of different organisms (McGuigan *et al.*, 1999, Smith *et al.*, 2000), and is a low-cost method suited to developing countries, where solar intensities tend to be high. However, due to repair mechanisms which bacteria have developed against photolytic treatment, there is a concern over whether solar disinfection has a lasting effect. Studies have shown regrowth of samples in the dark after irradiation is ended (Gelover *et al.*, 2006, Rincón & Pulgarin, 2004a).

Advanced Oxidation Processes (AOPs) achieve disinfection via the production of highly oxidising radicals. One such process is photocatalysis. The use of titanium dioxide (TiO_2) photocatalysis for disinfection has been subject to intensive research since TiO_2 was shown to have a strong bactericidal effect when under illumination (Matsunaga *et al.*, 1985). While other types of photocatalyst have been studied, TiO_2 is widely regarded as the most attractive photocatalyst for drinking water treatment due to its high efficiency and because it does not yield toxic by-products (Blanco & Malato, 2003, Ibáñez *et al.*, 2003, Robert & Malato, 2002).

It also has the advantages of being low-cost, widely available and photochemically stable (Paleologou *et al.* 2007).

In comparison with solar disinfection, TiO₂ photocatalysis has been seen to show encouraging residual disinfection characteristics. In the studies mentioned above, photocatalysis has been shown to completely inactivate bacteria, with no post-irradiation regrowth (Gelover *et al.*, 2006, Rincón & Pulgarin, 2004a). It is thought that the action of the reactive oxygen species completely inactivates the cell, which prevents subsequent regrowth. This is in contrast to solar disinfection, whereby the effects of DNA damage can be reversed by the target organism. It must be noted that some studies have shown that solar disinfection can also lead to further post-irradiation inactivation (Berney *et al.*, 2006a), although photocatalysis is widely thought to have a greater effect in this regard.

The majority of research in the area of photocatalytic disinfection has been conducted using *Escherichia coli* as the indicator organism. As this is not as resistant to treatment as some species (Blanco-Galvez *et al.*, 2007, Berney *et al.*, 2006), different types of bacteria (Block *et al.*, 1997, Ibáñez *et al.*, 2003) as well as viruses (Sjogren & Sierka, 1994, Laot *et al.*, 1999), fungi (Sichel *et al.*, 2007, Lonnen *et al.*, 2005) and protozoa (Dunlop *et al.*, 2008, Mendez-Hermida *et al.*, 2007) have also been researched and solar photocatalysis has been shown to be more effective than solar disinfection in the majority of comparative studies.

The use of distilled or sterile water in experiments has been shown to apply starvation (Berney *et al.*, 2006b) and osmotic (Sichel *et al.*, 2007) stresses on cells, meaning that results from experiments using distilled water should not be directly applied to practical applications, where waters may contain more ions and nutrients which would aid bacterial survival.

In the literature examined, the majority of solar photocatalytic research has been undertaken in the presence of suspended TiO₂. Despite showing a generally higher efficiency than supported TiO₂, suspended catalyst requires expensive and time-consuming post-treatment separation. Thus, reactors in which a fixed catalyst is employed would be a much more sustainable solution for water treatment in developing countries.

Although some research has been conducted into different fixed TiO₂ supports (as summarized by Bideau *et al.*, 1995), a standard cylindrical support is employed in the majority of studies in solar photocatalytic experiments for disinfection (McLoughlin *et al.*, 2004, Fernández *et al.*, 2005, Sichel *et al.*, 2007b, Gomes *et al.*, 2009). It is thought that this design could be improved in order to increase disinfection rates.

1.2 Aims & Objectives

The aims of the work presented in this thesis were as follows:

- To investigate the influence of UV treatment on *E. coli* disinfection rates in the absence and presence of catalyst.
- To investigate the effect of solar photolysis and solar photocatalysis on *E. coli*.
- To examine post-irradiation *E. coli* behaviour after treatment with and without catalyst.
- To determine whether the type of water used in disinfection experiments has a major effect on inactivation rates and post-irradiation events.
- To determine, using microscopy, the mechanism of cell inactivation brought about by UV light, again with and without photocatalyst.
- To explore the significance of interrupted illumination patterns on the inactivation rate and post-irradiation viability of bacteria.
- To investigate the effectiveness of photolysis and photocatalysis against a variety of bacteriophages and determine their suitability as indicators in research.
- To design a more efficient fixed photocatalytic insert geometry for use in compound parabolic collectors, using computational fluid dynamics (CFD).

1.3 Scope of Thesis

The initial stage of the thesis involves a study and review of relevant literature, paying particular attention to the solar photocatalytic disinfection of organisms.

The experimental section begins with an investigation into photolytic and photocatalytic bacterial inactivation rates under artificial UV light and natural sunlight. Post-irradiation events in the dark after photolysis and photocatalysis are also examined, while a microscopic study of target organisms is undertaken in an attempt to better understand the cell inactivation mechanisms of UV treatment in the absence and presence of catalyst.

A detailed analysis of the effects of different illumination times and patterns is then presented, with experiments conducted in a compound parabolic collector (CPC) under artificial UV light. Experiments were conducted in which the number of reactor tubes receiving illumination was sequentially reduced, as well as experiments where the net illuminated area was kept constant and the number of periods in light and dark per traverse was increased.

The applicability of photolysis and photocatalysis to the disinfection of viruses is investigated, with a study of three different bacteriophages under both artificial ultraviolet irradiation and natural sunlight. The suitability of the studied phages as indicator organisms was analysed, with reference to the comparative disinfection characteristics of *E. coli*.

As mentioned in Section 1.1, fixed catalysts are currently less efficient to those in suspension, due to factors such as the contact area available for photocatalysis and the distance between target organisms and photocatalyst particles. To this end, a regime of computational fluid dynamics (CFD) modelling was conducted to compare the efficiencies of a variety of insert geometries.

2. LITERATURE REVIEW

2.1. Water & Public Health

For centuries the cause of common diseases went unknown, meaning that epidemics could spread quickly. More recently, the discovery that microorganisms are the root of common infectious diseases has led to the eradication of many of these diseases. From a medical point of view, the development of antibiotics on a large scale in the mid-twentieth century helped to control many pathogenic bacteria. A second approach to controlling diseases has been the engineering of public health systems, which act by adjusting the environment to prevent the spread of pathogens. One of the major transportation routes for pathogens is water, so water treatment is at the heart of the environmental engineering approach to disease control.

Although improved health and sanitation systems have been developed to cope with most of the obvious pathogens, the increased populations will only stimulate the development of different pathogens, better suited to the new environment. All organisms compete with each other for nutrients, habitats, etc., so when one pathogenic microorganism is eradicated, another can come to the fore. As such, environmental engineers must constantly adapt their approaches to face the new challenges.

In developing countries, where sanitation is poor or non-existent, water-related diseases flourish, accounting for millions of lives a year. The United Nations set out in its Millennium Development Goals the target to 'Halve, by 2015, the proportion of people without access to safe drinking water and basic sanitation' (UN, 2000). By implementing sustainable water treatment systems in developing countries, it is hoped that this target can be met and subsequently improved upon.

2.1.1 Classification of Water-Related Illnesses

Whereas biologists classify water-related diseases by associating them with viruses, protozoa, bacteria, fungi, helminths, etc, an environmental classification system was developed based on the method of transmission of diseases. This system was developed by David Bradley (Lonholdt, 2005) and the four categories are:

- Waterborne
- Water-washed
- Water-based
- Water-related insect vector

The infections themselves are grouped in a slightly different manner, in the following categories: Faecal-oral, water-washed, water-based and water-related insect-vector. All water-borne diseases and most water-washed diseases can be transmitted by the faecal-oral route, which involves faecal matter being transferred to the mouth.

2.1.1.1 *Waterborne pathogens*

Waterborne diseases are caused by contamination of water from human or animal waste and contracted by the ingestion of this water (Hassan et al., 2005). It is important to remember that all infections transmitted by the water supplies may also be directly transmitted from faeces to mouth, or via contaminated food (Bradley, 1980, McGranahan, 2001). The most effective preventative measures against water-borne pathogens are the implementation of adequate water treatment systems and supply networks which do not allow for recontamination. By far the most common waterborne disease is diarrhoea, which can be caused by many different bacteria.

2.1.1.2 *Water-washed*

Water-washed diseases are diseases whose transmission is reduced by the use of greater quantities of water, almost regardless of the quality of the water. These diseases include diarrhoeal diseases, which are faecal-oral, and eye and skin infections, which are not (Howsam & Carter, 1996). The F-Diagram in Figure 2.1 illustrates the various measures that can be employed to control the spread of waterborne and water-washed diseases transmitted via the faecal-oral route. As can be seen, the primary barrier to spread of disease is the implementation of adequate sanitation.

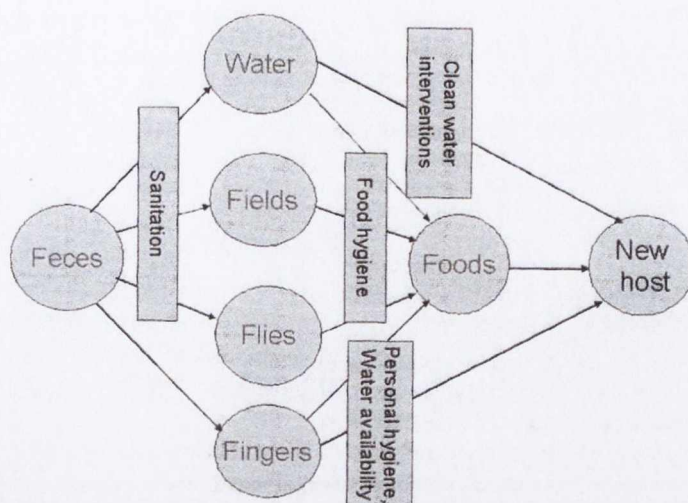


Figure 2.1 F-Diagram (Lonholdt, 2005)

2.1.1.3 *Water-based*

Water-based diseases require an intermediate aquatic host to transport the pathogenic bacteria or worms. The main such disease is schistosomiasis, where the pathogenic worm spends part of its life cycle in an aquatic snail host. Another water-based disease is Guinea worm, the only infection exclusively transmitted in drinking water (Caincross & Feachem, 2000).

2.1.1.4 *Water-related insect vector*

These are diseases such as malaria and dengue fever, which are transmitted by insects, especially mosquitoes, which have a larvae stage in water. The main method for controlling these diseases is to ensure sufficient drainage, therefore preventing stagnant pools of water, which provide a good home for these insects (see Table 2.1).

Water-borne	Improve quality of drinking water Prevent casual use of unprotected sources
Water-washed (or water-scarce)	Increase water quantity used Improve accessibility and reliability of domestic water supply Improve hygiene
Water-based	Reduce need for contact with infected water ¹ Control snail populations ¹ Reduce contamination of surface waters ²
* Water-related insect vector	Improve surface water management Destroy breeding sites of insects Reduce need to visit breeding sites Use mosquito netting

¹Applies to schistosomiasis only.

²The preventive strategies appropriate to the water-based worms depend on the precise life-cycle of each (see Appendix C) and this is the only general prescription that can be given.

Table 2.1 Transmission methods and main preventive strategies of water-related diseases (Cairncross & Feachem, 2000)

[Fewtrell *et al.* (2001) suggest adding a fifth category to this list, water-collection-related diseases, covering the possible infection of water during the journey to and from the source. They cited an outbreak of meningococcal disease in a refugee camp as an example of this.]

Table 2.2 lists the main diseases associated with each of the transmission routes described above.

Category	Infection	Pathogenic agent
1. Faecal-oral (water-borne or water-washed)	Diarrhoeas and dysenteries	
	Amoebic dysentery	P
	Balantidiasis	P
	<i>Campylobacter</i> enteritis	B
	Cholera	B
	Cryptosporidiosis	P
	<i>E. coli</i> diarrhoea	B
	Giardiasis	P
	Rotavirus diarrhoea	V
	Salmonellosis	B
	Shigellosis (bacillary dysentery)	B
	Yersiniosis	B
	Enteric fevers	
	Typhoid	B
	Paratyphoid	B
	Poliomyelitis	V
	Hepatitis A	V
	Leptospirosis	S
2. Water-washed:		
(a) skin and eye infections	Infectious skin diseases	M
	Infectious eye diseases	M
(b) other	Louse-borne typhus	R
	Louse-borne relapsing fever	S
3. Water-based:		
(a) penetrating skin	Schistosomiasis	H
(b) ingested	Guinea worm	H
	Clonorchiasis	H
	Diphyllobothriasis	H
	Fasciolopsiasis	H
	Paragonimiasis	H
	Others	H
4. Water-related insect vector		
(a) biting near water	Sleeping sickness	P
(b) breeding in water	Filariasis	H
	Malaria	P
	River blindness	H
	Mosquito-borne viruses	
	Yellow fever	V
	Dengue	V
	Others	V
B = Bacterium R = Rickettsia H = Helminth S = Spirochaete P = Protozoon V = Virus M = Miscellaneous		

Table 2.2 Common water-related diseases (Cairncross & Feachem, 2000)

2.1.2 Major Water-Related Diseases

2.1.2.1 *Diarrhoeal diseases*

Diarrhoeal diseases claim more lives than any other water-related disease, with an estimated 6,000 mortalities per day (UN, 2005). There are a reported 4 billion cases of diarrhoeal diseases a year, with the majority of deaths occurring in infants under the age of 5 (World Bank, 2003). Diarrhoeal diseases are caused by a wide range of microorganisms, including bacteria, viruses and protozoa. Species causing bacterial enteritis include *E. coli*, *Shigella*, *Campylobacter*, *Salmonella* and *Yersinia enterocolitica* (Cairncross & Feachem, 2000). Viral diarrhoea is primarily caused by *Rotavirus*, *Norwalk agent* (Hunter, 1997), while the term diarrhoea is also applied to protozoan diseases such as Giardiasis, Balantidiasis and Cryptosporidiosis, caused by *Giardia lamblia*, *Balantidium coli* and *Cryptosporidium*, respectively. As water-borne and water-washed diseases, these can be reduced by the introduction of appropriate disinfection techniques.

2.1.2.2 *Malaria*

Malaria is one of the most common water-related diseases (see Table 2.3) and is transmitted by mosquitoes, more specically those of the species *Anopheles*. The disease is caused by protozoan parasites, listed previously, and the tertian form of the disease is extremely dangerous, with a high mortality rate. The parasites and mosquitoes have developed a resistance against insecticides and drugs, respectively, meaning there is an ongoing challenge to prevent the spread of the disease (Ambasht *et al.*, 2003). As a disease transmitted by insect-vector routes, malaria cannot be controlled by disinfection methods. With no cheap and highly effective vaccines available, preventative measures need to be employed. Insecticides can be used to kill adult mosquitoes, while larvicides can be applied to breeding sites. Many insecticides, however, are becoming unpopular due to their environmental impact, so other methods are being researched, such as the draining of breeding sites and the use of mosquito nets.

2.1.2.3 Dengue Fever

Also transmitted by mosquitoes, in this case *Aedes aegypti*, dengue fever is a viral disease which occurs predominantly in densely populated areas. It is thought to afflict 50 million people a year and has a mortality rate of 5% (Clapham, 2004).

2.1.2.4 Schistosomiasis (Bilharzia)

Schistosomiasis is a disease which is becoming more and more widespread, mainly due to changes in the environment. For example, the construction of the Aswan High Dam in the Nile Delta led to infection rates of almost 100% in some areas (De Villiers, 2001). This is because the snail, which is a key part of the schistosome's life cycle, thrives in such areas. These parasitic flukes develop in the human bloodstream and are released to water, where they invade snails and grow. They are re-released to the water as cercariae, which can burrow through skin when they come into contact with humans.

Sickness (for detail see: www.cdc.gov/ncidod/dpd)	Incidence (cases/persons infected) million per year	Pathogen	Main water related transmission routes
Diarrhoea	4074	Bacteria	Water washed
		Virus	
		Protozoa	Water borne
		Helminths	
Malaria	214	Protozoa	Insect vector
Bilharzia (Schistosomiasis)	208	Helminth	Water based
Hookworm	152	Helminth	Water washed
Dengue fever	n/a	Virus	Insect vector
Roundworm (Ascariasis)	62	Helminth	Water washed
Japanese encephalitis	58	Virus	Insect vector
Whipworm (Trichuriasis)	45	Helminth	Water washed
River blindness (Onchocerciasis)	5.8	Helminth	Water based

Table 2.3 Incidence of main water-related diseases (Lonholdt, 2005)

2.1.3 Disinfection Methods

Disinfection is the destruction of pathogenic microorganisms in water. A good disinfectant is characterised by the following traits:

- Effective kill of pathogenic microorganisms
- Non-toxic to humans or domestic animals
- Non-toxic to aquatic species
- Easy and safe to store, transport and dispense
- Low cost
- Easy and reliable analysis in water
- Provides residual protection in drinking water

(Droste, 1997)

Disinfection can be achieved through the use of

- *Chemical agents*: These include chlorine and its compounds, bromine, iodine, ozone, phenol, dyes, soaps and acids.
- *Physical agents*: These involve the use of heat, light and sound waves, for example pasteurisation and UV treatment.
- *Mechanical means*: Microorganisms may be removed using coarse and fine screens, grit chambers and sedimentation tanks.
- *Radiation*: Gamma rays have also been used for water disinfection, although there are no full-scale installations in operation using this technology.

(Metcalf and Eddy, 2003)

The main disinfection methods are compared in Table 2.4 and a selection of the more common methods is described in more detail below.

Characteristic ^a	Chlorine	Sodium hypochlorite	Calcium hypochlorite	Chlorine dioxide	Ozone	UV radiation
Availability/cost	Low cost	Moderately low cost	Moderately low cost	Moderately low cost	Moderately high cost	Moderately high cost
Deodorizing ability	High	Moderate	Moderate	High	High	na
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	na
Interaction with extraneous material	Oxidizes organic matter	Active oxidizer	Active oxidizer	High	Oxidizes organic matter	Absorbance of UV radiation
Noncorrosive and nonstaining	Highly corrosive	Corrosive	Corrosive	Highly corrosive	Highly corrosive	na
Nontoxic to higher forms of life	Highly toxic to higher life forms	Toxic	Toxic	Toxic	Toxic	Toxic
Penetration	High	High	High	High	High	Moderate
Safety concern	High	Moderate	Moderate	High	Moderate	Low
Solubility	Moderately	High	High	High	High	na
Stability	Stable	Slightly unstable	Relatively stable	Unstable, must be generated as used	Unstable, must be generated as used	na
Toxicity to microorganisms	High	High	High	High	High	High
Toxicity at ambient temperatures	High	High	High	High	High	High

Table 2.4 Characteristics of the most widely used disinfection methods (Metcalf and Eddy, 2003)

2.1.3.1 Chlorination

Chlorination is by far the most commonly used method of disinfection. A number of chlorine compounds can be added to water to achieve disinfection, including chlorine gas (Cl_2), sodium hypochlorite (NaOCl), chlorine dioxide and calcium hypochlorite [$\text{Ca}(\text{OCl})_2$].

It is not without its problems, however, as it is difficult to achieve the optimal dose of chlorine due to a complex series of reactions that take place in the generation of free chlorine (Andersen, 2005). The ‘breakpoint’ concentration must be achieved, whereby enough chlorine is added to react with all oxidisable substances in the water, such that any extra chlorine added remains as free chlorine.

Hypochlorous acid is the most effective of the chlorine compounds in the destruction of pathogens. The hypochlorous acid reacts with unsaturated carbon-carbon bonds to form addition products and block further chemical reactions at this location. These unsaturated carbon-carbon bonds are essential to bacteria metabolism and so the addition reaction stops bacterial growth. This reaction does not kill the bacteria and is reversible, so a second step to the process is required. The addition of extra chlorine leads to the formation of

chloride ions, which break the carbon-carbon bond by oxidation and lead to cell death (McKinney, 2004).

Despite it being so widely used, there are some significant disadvantages to the chlorination process. Also, chlorine is a highly toxic substance in its natural form and is transported mainly by road and rail. Some disinfection byproducts (DBPs) of the chlorination process, especially the trihalomethanes (THMs), are known to be carcinogenic, while residual chlorine in treated water is toxic to aquatic life.

2.1.3.2 Ozonation

Ozone is an unstable gas formed when molecules of oxygen dissociate into single atoms. It can be produced by electrolysis, radiochemical reactions and photochemical reactions (Asano *et al.*, 2007). Ozonation systems generally involve passing air through a high voltage electrical discharge. The ozone is then pulled into the water to be treated via a vacuum created by a Venturi throat. Post treatment is required to remove insoluble metal oxides formed during the process. It is believed that disinfection by ozonation occurs via direct cell lysis (Asano *et al.*, 2007)

A major problem associated with ozonation is that it is highly energy-intensive. According to McKinney (2004), only about 5% of the total energy used to produce ozone is actually used in the process. If an adequate energy supply is available, however, ozonation can be an effective alternative to chlorination. As an extremely reactive oxidant, ozone is believed to kill bacteria through direct attack on the cell wall. Ozone is also generally considered to be more effective against viruses compared to chlorine (Metcalf & Eddy, 2003). Ozone must be generated on-site, as it breaks down to oxygen readily. As with chlorination, ozonation has harmful associated byproducts, especially if bromide is also present. Although THMs are not produced by ozonation, other byproducts such as various acids and aldehydes can be created.

2.1.3.3 Artificial UV Radiation

UV radiation has long been recognised as an effective disinfectant, targeting the DNA and RNA within microorganisms. Light in the germicidal range (~254nm) is known to cause

adjacent thymine bases on a DNA strand within a microorganism to form a thymine dimer (Figure 2.2). This blocks protein synthesis and prevents proper replication and transcription during the cell division cycle (Blake *et al.*, 1999, McDonnell, 2007). Viruses tend to be more resistant than bacteria to UV radiation, although most viruses are treatable using the method (Field *et al.*, 2003).

For UV radiation to be used effectively as a disinfectant, the water needs to be clear enough to allow the light to pass through. It should therefore follow some form of physical pre-treatment, removing the majority of the turbidity in the water (Field *et al.*, 2003).

2.1.3.3.1 *UV Lamps*

In the disinfection of water using UV, three main types of lamp are used: low-pressure low-intensity, low-pressure high-intensity and medium-pressure high intensity.

Low-Pressure Low-Intensity

These lamps have a broad radiation spectrum, but peak dramatically at 254 nm, with approximately 85 - 88 % of the output at 254 nm, making such lamps efficient disinfectants (Asano *et al.*, 2007). The low operating temperature, in the region of 40 °C, means that these lamps have a longer lifespan to higher intensity designs.

Low-Pressure High-Intensity

These lamps are similar to low-pressure low-intensity lamps, but the use of a mercury-indium amalgam as opposed to mercury gives them a far greater output.

Medium-Pressure High-Intensity

These lamps operate at temperatures of 600 – 800 °C, and generate roughly 50 to 100 times the UV-C output of low-pressure lamps (Asano *et al.*, 2007). These are the most effective lamps, but are more expensive and require more safety measures than the previous two types discussed.

2.1.3.3.2 UV system configurations

UV disinfection can take place in both open and closed channel systems. Low-pressure lamps tend to be employed in open channel flow regimes, while medium-pressure lamps are more usually employed in closed channel flow.

Open channel systems

In open channels, modules of UV lamps are arranged either horizontally or vertically in the system and the flow level is regulated to ensure that the lamps are constantly submerged. The lamps must be cleaned occasionally due to particle build up on their surface, which has a negative impact on performance.

Closed channel systems

In closed channel systems, lamps are generally placed perpendicular to the direction of flow. Due to the difficulty of access to the lamps, self-cleaning systems are generally implemented to maintain disinfection efficiency.

2.1.3.3.3 *Advantages and disadvantages of UV disinfection*

As UV disinfection is a physical process, it tends to be safer than chlorination and ozonation and does not lead to the formation of toxic byproducts. It also means that the dissolved solids content of treated water is not increased. However, despite the ever-improving technology, UV disinfection is still relatively expensive due to high operating costs – see Table 2.4 - and the design of the hydraulics of such systems is quite complex, as it is critical to performance.

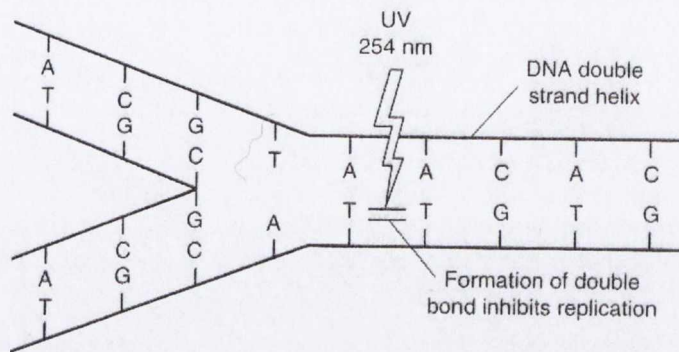


Figure 2.2 Formation of double bonds in UV-treated microorganisms (Metcalf & Eddy, 2003)

2.1.3.4 Pasteurisation

Pasteurisation involves disinfection through heating. The fact that water can be disinfected by boiling has been known for a very long time and this method is often still employed in individual cases. This cannot be done economically on a large scale due to the energy requirements, but is often used in emergency situations or on a house-by-house basis. Lower temperatures can be used for pasteurisation. Above 50 °C, the exposure time required for disinfection decreases exponentially, as can be seen in Figure 2.3. Heating systems for disinfection tend to be limited by the fact that some microorganisms can survive up to temperatures of 60 °C (Block, 2001) as well as the high costs associated with them. They do, however, achieve the eradication of a large variety of pathogens independent of parameters such as pH and turbidity, which adversely affect other disinfection methods (Burch & Thomas, 1998)

Solar heating has been studied as a possible disinfection method in developing countries. A simple schematic of a solar heating system is shown in Figure 2.4. Water is fed from a reservoir through a heat exchanger before entering a solar collector. After passing through this, its temperature is ascertained and, if the temperature is high enough, the water is allowed back through the heat exchanger into a reservoir containing treated water (Duff & Hodgson, 2005). Joyce *et al.* (1996) conducted laboratory experiments in which the temperature effects experienced by 2L plastic bottles containing water under Kenyan sunlight were replicated and

found complete disinfection of high levels of *E. coli* after 7 hours, with no sign of bacterial recovery 12 hours after the experiment. Solar pasteurization is regarded as a high cost solution for developing countries (Burch & Thomas, 1998), although more efficient, low maintenance designs are being developed (Duff & Hodgson, 2005).

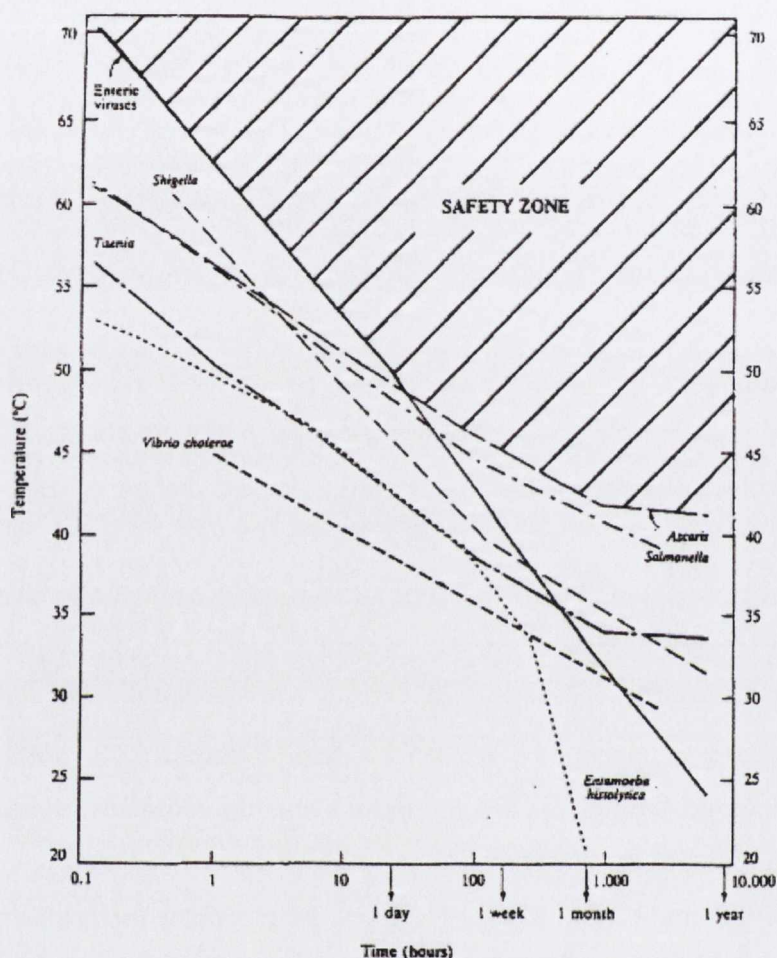


Figure 2.3 Graph relating exposure time to temperature required for pasteurization (Feachem *et al.*, 1983)

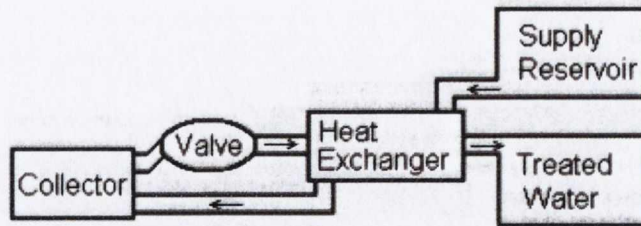


Figure 2.4 A simple continuous flow solar pasteurisation process (Duff & Hodgson, 2005)

2.1.4 Water storage

In order to ensure an adequate supply of water when it is required, a water storage system must be implemented to hold water post-treatment. Storage facilities must be constructed in such a way as to avoid re-infection of the water. In malarial areas, for example, uncovered pools of stagnant water attract populations of mosquitoes. Tanks for storage of treated water should therefore be covered and should also be constructed away from any source of pollution, such as livestock.

2.2. Microbiology of pathogenic organisms

2.2.1 Pathogenic Microorganisms

Water-related diseases have had a major effect on controlling human populations. There are many different types of pathogens that cause these diseases, including viruses, protozoa, bacteria, fungi and helminths (worms).

2.2.1.2 *Bacteria*

Bacteria are prokaryotic organisms, ranging in size from about 0.2 to 10µm. As prokaryotes, bacteria are distinguished from all other organisms (eukaryotes) by a number of features. The DNA molecules of bacteria are not separated from the cytoplasm by a nuclear membrane, but rather are arranged in a loop lying free in the cytoplasm (Kiely, 1996). The cytoplasm of prokaryotic cells is not split into different regions for specialised functions, for example respiration takes place on the cell membrane (Blake *et al.*, 1999).

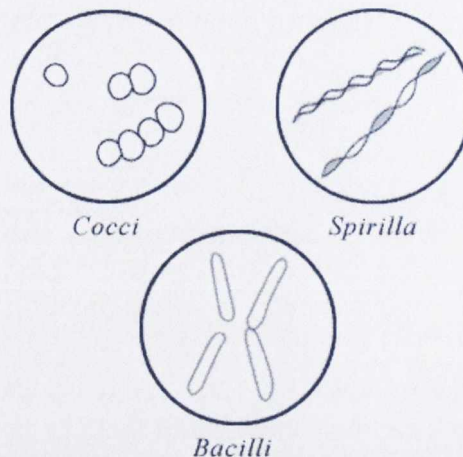


Figure 2.5 Basic bacteria shapes (www.cfsan.fda.gov/~dms/a2z-b.html)

There are three basic shapes of bacteria – cocci, bacilli and spirilla (Figure 2.5). All bacteria have the same general cell structure. From outside to in, one finds a slime layer, a cell wall, a cell membrane and the central cytosol (Figure 2.6). Cell walls consist of proteins, lipids and carbohydrates, while cell membranes are denser structures which control the ingress and egress of chemicals.

Bacteria are sub-divided into two categories, Gram-positive and Gram-negative, depending on the reaction of their cell wall to a specialised ‘Gram’ stain. Gram-positive bacteria possess a cell wall comprised of about 80 % peptidoglycan, as well as lipids, teichoic acids, proteins and polysaccharides. Gram-negative bacteria have a more complex cell wall, only about 10 % of which is peptidoglycan. Outside the thin (2 – 6 nm) peptidoglycan layer lies the outer membrane, 6 – 18 nm thick, which accounts for the rest of the cell wall. This consists of 50 % proteins, 25 % lipopolysaccharide and ~25% phospholipids (Kiely, 1996). The peptidoglycan and the outer membrane are responsible for providing the cell with mechanical protection, while also controlling the permeability of many molecules. This ability to control what enters the cell means that Gram-negative bacteria are usually more resistant than Gram-positive against many chemicals. Beneath the wall lies the cell membrane, or ‘cytoplasmic membrane’, which maintains the cell’s osmotic equilibrium by selectively regulating the transfer of certain metabolites. The cytoplasmic membrane contains protein enzymes that allow substrate energy transfer to intracellular enzymes, necessary for the synthesis of new cell components (McKinney, 2004). The cytoplasmic membrane is also important in DNA replication during

the cell division cycle and acts a binding site for bacterial DNA. The periplasmic space is found between the cell wall and cytoplasmic membrane and is responsible for processing any foreign bodies which have passed through the cell wall but are too large to pass through the cell membrane. As it is an area affected by the cell's external environments, it could play a very important role in cell defence (Blake *et al.*, 1999). The region within the cell membrane is called the cytoplasm, and is the medium in which metabolic processes take place (Winegardner, 1995). In prokaryotic cells, the cytoplasm is not differentiated into separate regions for certain functions, with the nuclear material being dispersed throughout the 'cytosol', which is the fluid that makes up most of the cytoplasm. Cytosol is a colloidal suspension of mainly proteins, which provide the building blocks for the cell's key enzymes. As mentioned above, the nuclear material is dispersed throughout the cytoplasm, with DNA existing as strands in the cytosol, surrounded by the RNA responsible for protein synthesis.

On the exterior of the cell wall, bacteria may have other structures such as slime layers, flagella, pili and cilia. The slime layer is a viscous coating which provides extra protection to a cell and facilitates attachment of bacteria to other structures. Flagella are protein strands which extend from the cytoplasmic membrane through the cell wall and can act as propellers to move the bacteria through a liquid medium. Pili are smaller protein projections that appear later in a cell's life cycle and also aid attachment of bacteria to other bodies. Protective coatings such as the slime layer are often formed in response to external stimuli and so can be absent in, for example, laboratory cultures and must be considered in the development of any treatment processes (Blake *et al.*, 1999).

Certain bacteria can also produce spores, which are believed to be the most resistant organisms to disinfection methods (McDonnell, 2007). This is thought to be due to the differences in the peptidoglycan between vegetative cells and spores (Barton, 2005) and the decreased permeability of spores to species such as hydrogen peroxide when compared with growing cells (Piggot *et al.*, 1994). In contrast with viruses, only a very small proportion of bacteria are pathogenic. The pathogenic bacteria can either be parasitic or toxin-producing. Parasitic pathogens rely on their host for nutrients and as bacteria numbers grow the host's nutrient supply is affected, leading to the eventual death of the host. Some parasitic bacteria limit their growth, meaning that the parasite and host can coexist. Toxin-producing bacteria produce protein compounds that are toxic to the host organism. As with viruses, the most

important pathogenic bacteria are enteric pathogenic bacteria, which are formed inside animals and spread via faecal wastes. One such disease is cholera, caused by the *Vibrio cholerae* bacteria, which is still endemic in many parts of the developing world in areas of poor sanitation. Another example is typhoid, which is caused by the *Salmonella typhi* bacteria.

In the mid-twentieth century, the large scale development of antibiotics helped to control a lot of the pathogenic bacteria present in the environment. As with all microorganisms, the demise of some strains of bacteria led to the rise of other, stronger pathogens, which were resistant to the antibiotics then in use. Although a common misconception is that new virulent pathogens were being formed, the truth is that they had always existed and were now just thriving due to lack of competition. Thus, the fight against pathogens is a continuous one.

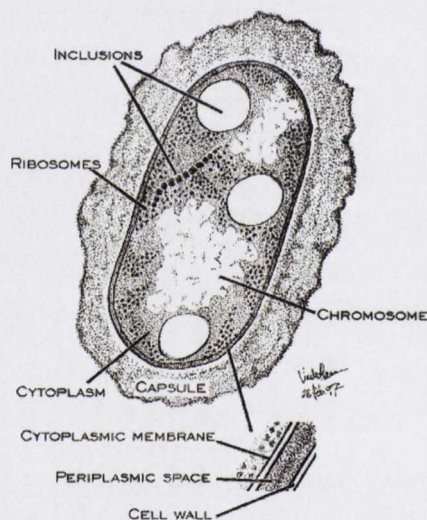


Figure 2.6 A simple bacterial cell structure (www.bact.wisc.edu/.../structure.html)

2.2.1.2 Viruses

Viruses are much smaller and more simple organisms than bacteria, ranging in size from 0.01 to 0.3 μm (Blake et al., 1999). They are unique in that they have no independent metabolic activities, rather they must infect living host organisms in order to reproduce, thus being

known as 'obligate intracellular parasites'. They do not respire and remain inert outside of host organisms. A virus consists of RNA or DNA (and not both), which carries the virus genome, contained in a protective protein coat, or capsid. The binding site for attaching a virus to a host is located on the protein coat of the virus. Some viruses have an external coat, or envelope, consisting of host-originated fats and proteins, which allow viruses to bind to the host and gain entry. Viruses attach themselves to host cells and inject their genetic materials into the cells' cytoplasm as the viruses are uncoated (Beckett, 1986). They use the host cell's mechanisms to replicate the virus genome and structural virus proteins are formed. The genome controls the assembly of new virus particles and these emerge through lysis of the infected cell, enabling them to move on to infect other cells (Mara & Horan, 2003). If the binding site of a virus is attacked by a disinfection method, it can no longer recognise receptor sites on host cells. Retroviruses, such as HIV, are peculiar in that although the virus particle contains an RNA genome, a DNA copy of it is made by an enzyme known as reverse transcriptase when the virus infects a host cell. The DNA copy subsequently becomes incorporated into the genome of the host cell.

Despite their relatively simple structure, there are many different shapes of virus, although they are subdivided into three basic categories: helical, polyhedral, enveloped and complex (Tortora *et al.*, 2007). Helical viruses contain a strand of nucleic acid within a helical capsid and include the Ebola virus. Polyhedral viruses are predominantly icosahedral in shape, the capsid consisting of twenty triangular faces and twelve corners. These include the adenovirus, which consists of an icosahedral capsid covered in spikes. Among the 47 known types of adenovirus, two are cited as important causes of gastrointestinal illness, while others are responsible for upper respiratory illnesses such as the common cold. Enveloped viruses, as discussed earlier, have an external coat outside of the capsid and tend to be spherical in shape. The influenza virus is an example of an enveloped helical virus, while the herpes simplex virus is an enveloped polyhedral virus. Complex viruses, such as the T-even bacteriophage in Figure 2.7, have a more complicated structure, with extra structures attached to the capsid.

When viruses grow rapidly, they can bring about a failure of the host organ and in turn the death of their host. Animals can, however, develop antibodies which provide them with immunity towards specific viruses. The most common virus transmitted through water tend to be enteroviruses (which are responsible for, e.g., polio, hepatitis), which are formed within

animals and spread to water via faecal contamination. Enteroviruses can be carried long distances by water and can be transferred to humans if said water is used for drinking or irrigation of crops. It has also been observed that shellfish concentrate enteroviruses, meaning there is cause for concern if they are harvested from areas where contaminated water drains into the ocean (McKinney, 2004). The nature of viruses means that the quick identification and treatment of new viruses is difficult.

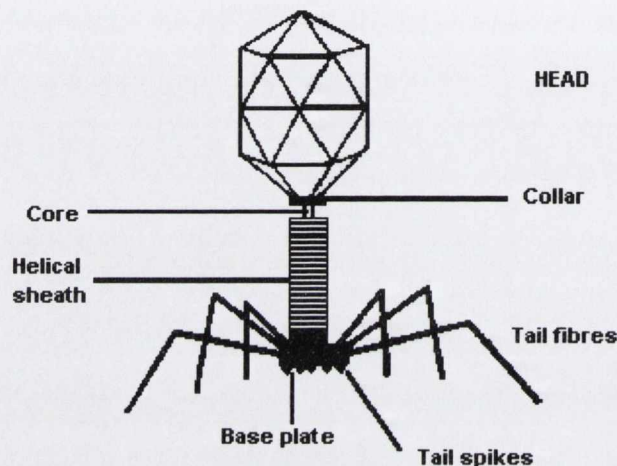


Figure 2.7 T-even bacteriophage (www.leeds.ac.uk)

2.2.1.3 *Fungi*

Fungi are aerobic, multi-cellular (with the exception of unicellular yeast) organisms that are found mostly in the soil but can also occur in sea and freshwater (Kiely, 1996). Fungi consist of a tangled mass of filaments called hyphae, which are partly subdivided into separate cells by walls, or 'septa' (Fried & Hademenos, 1998). The total filamentous mass is known as a 'mycelium' (Figure 2.8) and when this is in a fast-growing stage, a certain type of hyphae known as haustoria can appear. As eukaryotes, they contain a nucleus of genetic material surrounded by a membrane and their cytoplasm is segregated into distinguishable units which perform different functions. The cell wall is made up of multiple layers of fibrils, consisting of chitin as well as lipids and proteins. For food, fungi recycle essential plant nutrients and also infect hosts as parasites. Reproduction can occur by either the spreading of spores or by the fusion of the nuclei of two parent cells. Pathogenic fungi tend to be parasites, again using their host as a nutrient source. As they are aerobic microorganisms, fungi tend to be found in the blood and lungs of host animals. The more rigid cell wall structure of fungi means that they

tend to be more difficult to control than bacteria and can survive in a wider variety of environments. As both fungal and human cells are eukaryotic, most chemicals that kill fungi are also harmful to humans. Once a fungus has developed on a host organism, it is very difficult to control. It is thus fortunate that very few fungi are actually pathogenic and that water is a poor medium for the transport of fungi.

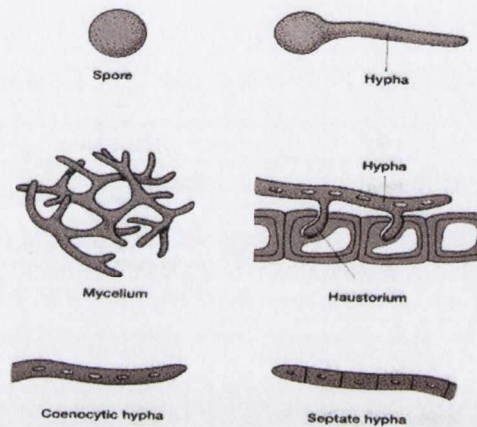


Figure 2.8 Constituents of fungi (kentsimmons.uwinnipeg.ca/.../lb2pg20.htm)

2.2.1.4 Protozoa

Pathogenic protozoa are parasitic, eukaryotic organisms and use the host as a source of nutrients. They are unicellular, lack a cell wall, and range in size from 1 to 150 μm (Cox, 1993). The cell membrane acts as the interface between the protozoan and the host and is covered with molecules which can either act as targets for immunological attack or as part of the immune evasion process (Davies, 1999). Locomotion is achieved in a number of ways. Amoebas have pseudopods, which are extensions of their cytoplasm, while other protozoa possess long flagella or shorter cilia. Protozoa reproduce asexually by fission, budding or schizogony (multiple fission), although sexual reproduction by conjugation has also been observed in some species. Under certain adverse conditions, encystment may occur. This is the production of a protective shell, called a cyst, which allows the organism to survive in unfavourable conditions, such as in the absence of nutrients, in unsuitable temperatures and in the presence of harmful chemicals. Importantly, the production of a cyst also allows a usually

parasitic protozoan to survive without a host organism. Protozoa are usually aerobic heterotrophs, although some can grow anaerobically. All protozoa inhabit areas with a plentiful supply of water and have developed many different ways of consuming nutrients. Some transport food directly across their plasma membrane, but those with a protective pellicle have had to adapt. Ciliates use their cilia to direct their food to a mouthlike opening known as a cytostome, while amoebas surround it with their pseudopods and take it in via phagocytosis. The few protozoa that are pathogenic include *Giardia Lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica*. *Giardia lamblia* grows in small wild animals and its cysts are transmitted to water via faecal contamination. It is commonly transferred to humans if they drink untreated stream water in contaminated areas. Cryptosporidiosis is the disease caused by *Cryptosporidium parvum*, a coccidian member of the sporozoa, spore-producing protozoa. Humans become infected upon ingestion of its highly resistant oocysts (cysts formed in members of the phylum Apicomplexa), usually through drinking water. These release sporozoites into the small intestine, which can infect cells and produce further oocysts. The disease is a diarrhoeal one, not dissimilar to cholera, and can be life-threatening in immunodeficient patients. The most commonly known protozoan disease is malaria, which is caused by four different types of parasite: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*, *P. falciparum* and *P. vivax* being responsible for the majority of malarial diseases (Cairncross & Feachem, 2000).

2.2.1.5 Helminths (Worms)

Helminths, especially *Nematoda* (roundworms) and *Platyhelminthes* (flatworms), are a major concern in the tropics, leading to diseases such as schistosomiasis (bilharzias) and guinea worm infection. In some diseases, helminths produce eggs which can survive in water and infect humans through ingestion of this water. Some pathogenic helminths infect humans via an intermediate host. This is the case with schistosomes, which invade snails in the aquatic environment, are released as cercariae and infect humans by penetrating through the skin (Bannister *et al.*, 2000). Helminth eggs can be removed by water by sedimentation processes (Ujang *et al.*, 2006).

2.2.2 Indicator Organisms

Although the detection of various pathogenic bacteria, viruses and protozoa is possible using modern microbiological methods, it is still not practical to isolate and test each individual microorganism. Indicator organisms are used in microbiology to represent a wide range of biological contaminants. Many pathogens are difficult to detect and so cannot be tested for easily. A good indicator organism must fulfil a few criteria: it should be detectable by cheap and simple methods; it should have a lifespan similar to the pathogen under analysis; it should be present when the pathogen is present and not detectable when the pathogen is absent; it should not be able to multiply in the environment after having been shed by its host; it should have constant characteristics; and it should not be present in unpolluted water (Health Canada, 2006; Mitchell, 1978). Indicator organisms do not give definite representations of the abundance of the particular pathogen, but from the indicator organism analysis, probable pathogen concentrations can be inferred. Bacteria are often chosen as they follow easily reproducible growth patterns and reproduce very quickly due to their small size and simple metabolism.

2.2.2.1 *Escherichia coli*

E. coli is used as an indicator organism in many microbiological tests. It is a rod-shaped, Gram-negative bacteria, part of the family *Enterobacteriaceae*. It is found naturally in the intestines of warm-blooded animals, and, unlike other members of its family, does not usually occur naturally in soil, water or plants. *E. coli* rarely grows outside the gut of humans or animals and therefore its inability to grow in water means that the detection of *E. coli* in water is an indicator of recent faecal contamination.

2.2.2.2 *Escherichia coli* as an indicator organism in solar photocatalysis

Of the pathogens found in drinking water, it is those arising from faecal contamination which pose the greatest danger. This is why it is important to be able to determine the extent of faecal contamination. The Gram-negative *Escherichia coli* bacterium was isolated from a cholera patient in 1885 by Escherich, after whom it was named, and after further investigation,

Schardinger proposed in 1892 that *E. coli* could be used as an indicator of faecal contamination (Nollet, 2000). *E. coli* was found to be the only coliform bacteria found exclusively in the intestines of humans and warm-blooded animals and was also found to have a similar lifespan to other enteric bacterial pathogens. The fact that they do not multiply in the environment and are excreted in large numbers also contributes to their suitability as an indicator organism.

The majority of authors discussing photocatalytic disinfection of water have used *E. coli* as an indicator organism. It is, however, thought by some to be a poor indicator of real water systems, as it is not as resistant to irradiation damage as many pathogens (Blanco-Galvez *et al.*, 2007).

2.2.2.4 Bacteriophages as indicator organisms

As in the case of bacterial indicators, viral faecal indicators are chosen due to their resilience and association with human infections. The two most commonly used viral indicators are enteroviruses and rotaviruses, both of which are icosahedral viruses. Rotaviruses cause acute gastroenteritis and are responsible for the majority of cases of life-threatening diarrhoea in children under the age of 2 (Mara & Horan, 2003). Enteroviruses include polioviruses, coxsackieviruses and echoviruses and are found in wastewater and surface water (Asano *et al.*, 2007). Coliphages, whose hosts are *E. coli* cells, are also often used as indicators for human enteric viruses.

Bacteriophages are viruses which attack bacteria and are widely used as indicators of animal viruses and enteric bacteria. The advantages of using bacteriophages are that they are easily detected at low cost and phage assays take far less time than the quantification of enteric viruses (Goval *et al.*, 1987). The most common studied bacteriophages are those associated with *Escherichia coli* and are known as coliphages. They are considered important as their behaviour in nature gives an insight into the behaviour of human enteric viruses. Coliphages are obligate parasites, meaning that they require actively metabolizing bacterial hosts in order to reproduce. Typically, although the steps involved in replication vary from one phage to another, virulent coliphages attach themselves to specific receptor sites on *E. coli* cells and inject their DNA or RNA into the host, leaving the protein coat or capsid outside the

bacterium. The phage nucleic acid redirects the metabolism of the host to synthesise a number of enzymes. These enzymes destroy the bacterial nucleic acid and direct the synthesis of new phage nucleic acid and capsids. Mature phage particles are released from the infected cell, sometimes after induced cell lysis (Volk & Brown, 1997, Madigan & Martinko, 2006, Atlas & Bartha, 1997, Hurst *et al.*, 2007) .

2.2.3 Microbiology of water-related diseases

2.2.3.1 *Cholera*

Cholera is caused by *Vibrio cholerae*, a rod-shaped, Gram-negative bacterium which is transmitted via infected water. After ingestion of water containing more than approximately 10^6 such cells (Mara & Horan, 2003) the *Vibrio cholerae* attaches to epithelial cells in the small intestine and releases cholera enterotoxin (Note: the infectious dose becomes as low as 10^4 cells if antacids have been taken, as *Vibrio cholerae* is usually controlled by stomach acid). The enterotoxin increases the levels of cyclic AMP, which is a specific mediator of regulatory systems including ion balance. As a result, ion concentrations are altered and a large amount of water is released into the intestinal lumen. This water is lost at a greater rate than it can be absorbed by the large intestine and so dehydration results in the victim. Cholera is therefore treated by the intake of fluids containing electrolytes to restore the ion balance. Despite both being members of the same subdivision of proteobacteria, *Vibrio cholerae* and *Escherichia coli* are quite different, and are members of two different families, the Vibrionaceae and Enterobacteriaceae respectively. In a test comparing the solar disinfection of various enteric pathogens, *Vibrio cholerae* was shown to be far more sensitive to sunlight and to mild heat treatment than *E. coli* was, which suggests that *E. coli* is a safe indicator for *V. cholerae* (Berney *et al.*, 2006).

2.2.3.2 *Typhoid*

Typhoid fever is caused by *Salmonella typhi*, again a rod-shaped, Gram-negative bacterium. This can be transmitted by direct contact with infected persons and by the ingestion of contaminated food, but by far the most common means of transmission is via contaminated water. In the experiment mentioned in Section 2.5.4.1, *Salmonella typhimurium* was seen to be

far more resistant to solar treatment than *E. coli* was. If this holds for other strains of *Salmonella*, then *E. coli* would not be a suitable indicator organism for solar disinfection of *Salmonella typhi*.

2.2.3.3 Giardiasis

Giardiasis is an acute form of gastroenteritis caused by *Giardia intestinalis*, a flagellated protozoan. The cysts produced by the protozoan cells attach to the intestinal wall after ingestion of contaminated water, causing diarrhoea and nausea. The thick walls of the cysts resist normal disinfection methods such as chlorination and UV radiation alone, although if proper clarification and filtration is followed by one of these methods, cysts are usually removed. In isolated cases, water can also be filtered and boiled to remove the pathogen.

2.2.3.4 Cryptosporidiosis

The *Cryptosporidium parvum* protozoan is a parasite in a number of warm-blooded animals. The protozoan produces oocysts which are highly resistant to chlorination - up to fourteen times as resistant as are the *Giardia* cysts (Madigan & Martinko, 2006) – and to UV radiation, meaning that sedimentation and filtration methods are always used to remove *Cryptosporidium*. Cryptosporidiosis is a diarrhoeal diseases which usually subsides of its own accord after two weeks, but is dangerous when contracted by infants, old people, or people with impaired immunity.

2.2.3.5 Malaria

Malaria is caused by a member of the Sporozoa group of protozoa, with *Plasmodium falciparum* being the most serious of the four forms which infect humans. The disease is spread by females of the *Anopheles* species of mosquito, which inject sporozoites into the bloodstream. These are small cells which then travel to the liver, where they either remain quiescent or replicate and become enlarged before segmenting into merozoites. The merozoites are released from the liver into the bloodstream, infecting red blood cells and replicating as before.

2.3. Solar radiation

2.3.1 Sunlight and Ultraviolet Light

Light is an electromagnetic wave, which does not depend on an external medium. Electromagnetic waves are transverse waves covering a huge range of wavelengths, from gamma rays to radio waves. The visible light spectrum lies between 400 and 700 nm.

The sun radiates a huge amount of solar energy, the small fraction of it that reaches the Earth is by far our largest source of our energy. The solar spectrum is divided into a number of different bands based on wavelength.

The relatively minuscule fraction of the energy that reaches the exterior of the Earth's atmosphere is known as the solar constant, its value considered to be uniform enough for all practical purposes. The value of the solar constant is in the region of 1.4 kW/m^2 .

The advantages of using solar energy for environmental applications in the developing world are relatively obvious, it being a constant, free natural resource which does not lead to pollution. It is also extremely abundant in the vast majority of developing countries.

2.3.1.1 Atmospheric effect

As space is a vacuum, solar radiation does not experience scattering, absorption or reflection while travelling through it. The fraction that reaches the Earth, however, does experience these phenomena. About 30 % of solar energy, known as the planetary albedo, is reflected back into space by clouds and higher atmospheric layers and is unavailable to us. About 25 % is absorbed by gases, clouds and atmospheric particles, which remove energy from the beam and thus heat the atmosphere. Scattering occurs in two ways. Firstly, individual quanta can be diverted relatively uniformly in all directions. This process is called selective or Rayleigh scattering, named after the physicist who proved that the effectiveness of such scattering is inversely proportional to the fourth power of the wavelength of the light. Blue light, which has a wavelength of 400 nm is thus scattered about nine times as readily as red light, whose wavelength is 700 nm. This explains the blue colour of the sky when viewed from the ground.

At midday, the sun appears almost white, as very little scattering occurs when the sunlight is passing through the smallest thickness of atmosphere. At sunrise and sunset, the sunlight must travel through a far thicker layer of atmosphere, leading to more scattering, particularly of blue light, and thus the reddish appearance of the sun. The other mechanism is known as non-selective, or aerosol, scattering and is caused by larger diameter particles, such as dust, fog, smoke, pollen, etc. The amount of scattering is generally believed to be equal for all wavelengths and explains the white appearance of clouds and fog, which consist of colourless water molecules.

2.3.1.2 Ground level radiation

Due to the atmospheric effects outlined in Section 2.3.1, the intensity of the sunlight reaching the Earth's surface is much reduced, varies greatly for different wavelengths and depends on factors such as time of year, cloud cover, latitude and pollution levels. The radiation that reaches ground level consists of direct and diffuse radiation. Direct radiation, as the name suggests, is not altered in its path from the sun, whereas diffuse radiation is the result of atmospheric scattering and reflection from the Earth's surface. Diffuse radiation accounts for up to about 20 % of the total ground level radiation, although this value fluctuates greatly depending on time of day and cloud cover.

Ultraviolet radiation accounts for about 5 % of the light reaching us from the sun and is that part of the spectrum between 100 and 400 nm. Ultraviolet radiation is split up into UV-A (320-400nm); UV-B (280-320nm); and UV-C (100-280nm). UV-A is the most abundant ultraviolet light in sunlight, making up about 95% of the UV light. The solar spectrum is shown in Figure 2.9.

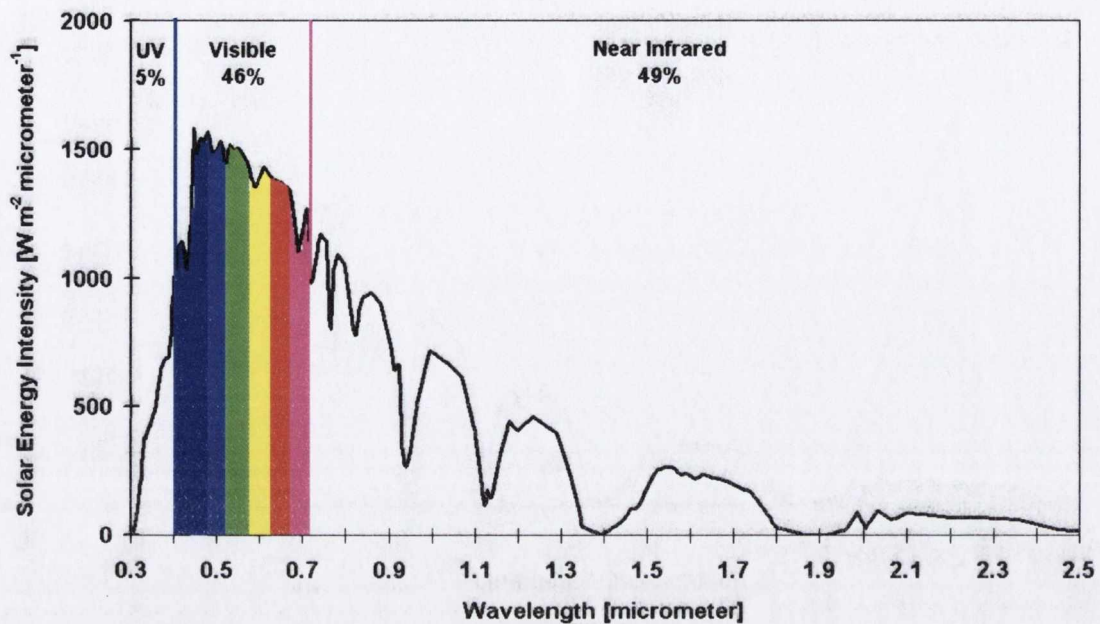


Figure 2.9 The solar spectrum (www. easthorse.net)

2.3.1.2.1 Standard reference spectra

Reference spectra are used as a benchmark by which to test different spectrally selective devices. Originally there were separate reference spectra for direct and global tilted irradiance, but as technologies changed and scientists realized that the direct spectrum could not reproduce practical conditions, it became practically obsolete (Muneer *et al.*, 2004). The current standard spectrum is G173-03, devised by the American Society for Testing and Materials (ASTM, 2003), which can be seen in Figure 2.10. Possible reference spectra are constantly evolving and a recent review was presented by Gueymard *et al.* (2006).

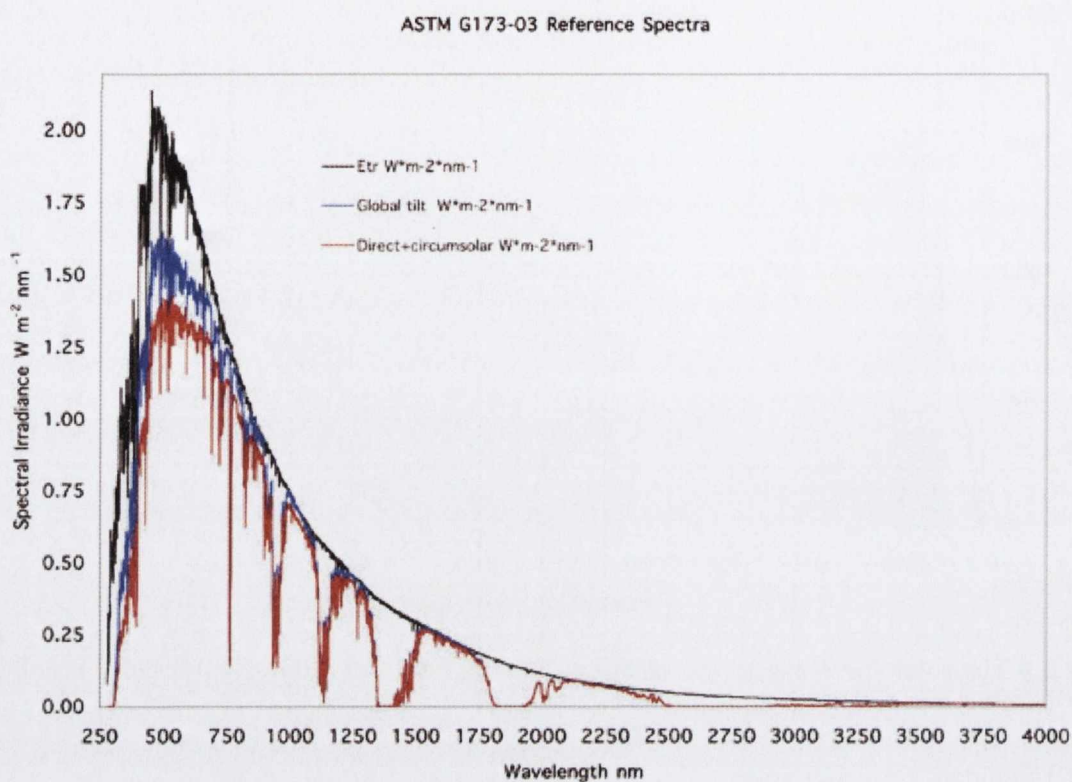


Figure 2.10 The standard reference spectrum (ASTM, 2003)

2.3.1.3. Suitability of regions for solar applications

In a paper of 1984, the earth was divided into hypothetical bands of favourability for solar energy applications, depending on the amount and intensity of sunlight received. The most favourable belts were described as those between the latitudes 15° and 35° , both north and south naturally. These areas receive by far the highest amount of sunlight, the direct proportion of which is about 90 %. The band around the equator is less favourable, receiving on average 2,500 as opposed to 3,000 hours of sunshine per year and having higher levels of humidity and cloud cover (Acra *et al.*, 1984b). The diagram of these belts is given in Figure 2.11.

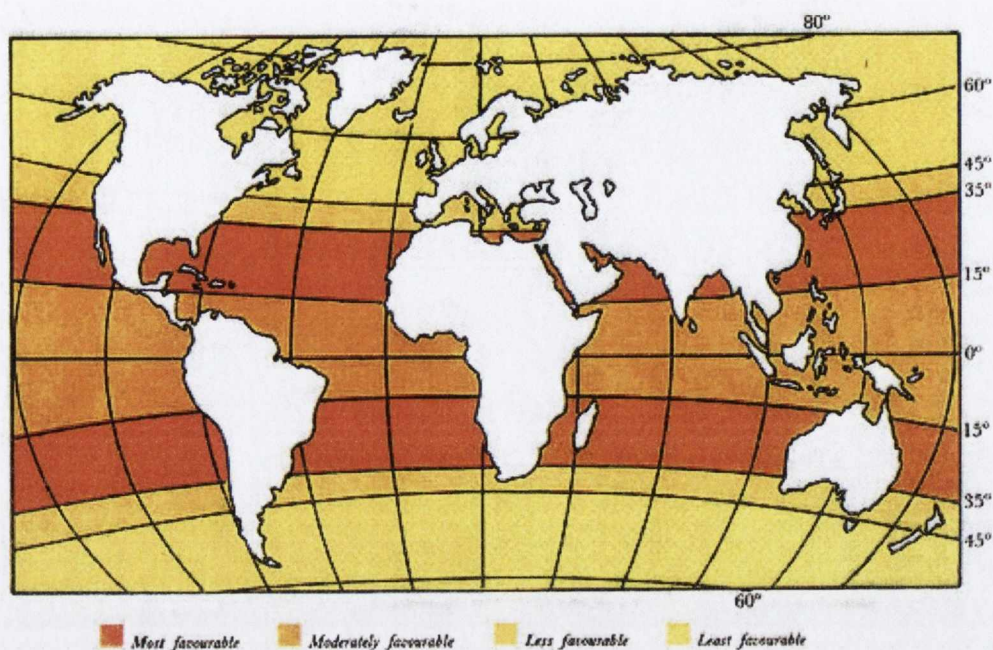


Figure 2.11 World distribution of solar radiation indicating suitability of areas for solar applications (Acra *et al.*, 1984b)

2.3.2. SODIS

Recently, the area of batch process solar disinfection (SODIS) has been heavily funded and researched in the hope that it will provide a solution to drinking water needs in developing countries. The scheme employs PET or PVA bottles, which are filled with water and left on roofs of buildings by the locals for a recommended period of time to be treated by a combination of the heat and UV-A irradiation from sunlight (Figure 2.12). At a temperature greater than 45° a synergy occurs, whereby the effect of temperature and UV is much greater than the sum of the single effects. The efficiency of the system can be improved if the bottles are left on sunlight-reflecting materials such as corrugated iron roofs. In areas where high concentrations of iron occur naturally, SODIS can be improved by the addition of H_2O_2 in order to facilitate the photo-Fenton reaction (Eqn. 2.11) (Sciacca *et al.*, 2010).

Health impact studies have been carried out in countries where such schemes have been implemented, and it has been found that cases of diarrhoea are cut by between 20 and 50 % when SODIS is used. SODIS has been proven to be effective against a variety of organisms, as

presented in Table 2.5. Of particular appeal is its ability to treat water containing protozoans such as *Cryptosporidium parvum*, which are resistant against a number of more common disinfection techniques. It was found that, while turbidity and exposure time are important factors for disinfection, solar intensity is the most significant parameter in the treatment of *Cryptosporidium parvum* by SODIS (Gómez-Couso *et al.*, 2009).

SODIS is a low-cost solution which is easily implemented, although it requires education of the users. Common mistakes cited by EAWAG are:

- The use of bottles of the wrong colour, which are not absorbent to the appropriate wavelengths of light.
- The use of bottles which are larger than the recommended 1-2 litres. The larger volume/surface area ratio means it takes much longer for the water to be treated.
- The placing of bottles upright, thus increasing the depth of the water.
- The transfer of treated water into contaminated vessels prior to consumption.

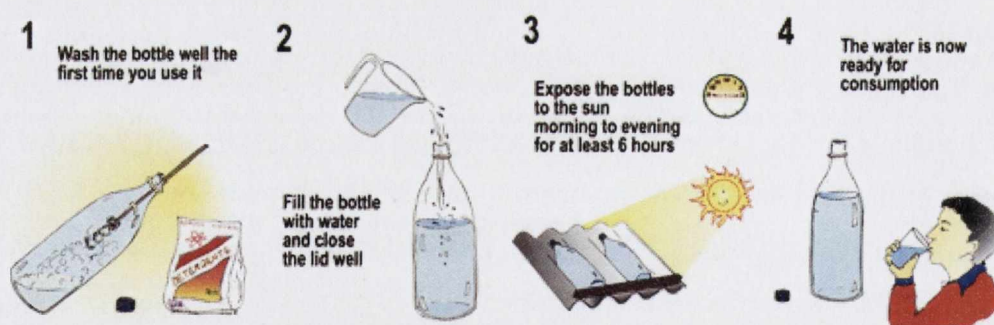


Figure 2.12 The SODIS method (www.sodis.ch)

The scheme also has its limitations. In an advisory pamphlet published by EAWAG, the following are cited:

- SODIS does not change the chemical water quality

- SODIS requires relatively clear water (< 30 NTU)
- SODIS is not useful for the treatment of large volumes of water
- SODIS requires suitable weather conditions

A recent review of the SODIS process determined the most important factors for the sustained use of SODIS and these focused on the continuous influence of respected, motivated promoters, the availability of bottles, social pressures, health effects being felt by users and the backing of schemes by local institutions (Meierhofer & Landolt, 2009).

The latest step in the development of SODIS is the introduction of a photocatalyst. A research team has been set up at the Royal College of Surgeons to test the effectiveness of SODIS and to develop some simple enhancement technologies for the method, such as UV dose indicators, photocatalytic bottles and continuous flow systems (<http://www.rcsi.ie/sodis>).

Pathogen	Illness	Reduction through SODIS ** at water temperatures of 40°C and solar exposure of 6 hours
Bacteria		
E.coli	Indikator for Water Quality & Enteritis	3-4 log (99.9 -99.99%)
Vibrio cholera	Cholera	3-4 log
Salmonella spp.	Thyphoid	3-4 log
Shigella spp.	Dysentery	3-4 log
Viruses		
Rotavirus	Diarrhoea, Dysentery	3-4 log
Polio Virus	Polio	inactivated, results not yet published
Hepatitis Virus	Hepatitis	Reduction of cases of SODIS users
Protozoa		
Giardia spp	Giardiasis	3-4 log (Infectivity of Cysts)
Cryptosporidium spp.	Cryptosporidiasis	2-3 log (Infectivity of Cysts)

Table 2.5 Reduction of microorganisms by SODIS (www.sodis.ch)

2.4. Solar disinfection

2.4.1. Background to solar disinfection

The use of sunlight to treat water is by no means a new phenomenon, according to Conroy *et al.* (1996) exposure to sunlight has been used as a means of water purification for as long as 4000 years. Downes and Blunt (1877) linked the effectiveness of sunlight to a bactericidal effect. They reported that test tubes containing Pasteur's solution left in the sunlight developed no bacteria, while test tubes shielded from the light did. They went into more detail by investigating the effect of different wavelengths, discovering that more contamination was found in test tubes left in red and yellow glass boxes than in blue and clear glass, leading them to suspect that UV light was responsible for disinfection. The use of UV light was a major breakthrough in water disinfection, due to its relative low cost and energy needs. The first communal UV sterilisation system was installed in 1910 in Marseille, treating pre-filtered water from the River Durance.

The use of sunlight to disinfect water on a household scale in developing countries was first documented in an instructional document in 1984 (Acra *et al.*, 1984a). This was aimed at the 'housewives in rural areas of developing countries where safe community water supplies are not available' and outlined how water should be left in clear bottles and exposed to sunlight to achieve disinfection. This formed the basis of the SODIS project, which is discussed in Section 2.3.2.

Recently, there has been a lot of research into the area of Advanced Oxidation Processes (AOPs), including SPC-DIS (solar photocatalytic disinfection), which are used to produce strong oxidative agents. The most effective of these so far is photocatalysis, which is defined as the acceleration of a photoreaction in the presence of a catalyst (Sichel *et al.*, 2005). More specifically, the use of TiO₂ photocatalysis for disinfection has been under investigation ever since Matsunaga and co-workers reported the complete sterilisation of *lactobacillus coli* when subjected to UV-irradiation in the presence of platinum-loaded TiO₂ (Matsunaga *et al.*, 1985).

TiO₂ photocatalysis is a very attractive method of water disinfection for a number of reasons. It is affordable, does not produce hazardous byproducts and has been found to be effective

against microorganisms which are resistant to most disinfection methods, such as *Cryptosporidium parvum* (Méndez-Hermida *et al.*, 2007).

2.4.2 Mechanism of solar disinfection

UV, red and infrared radiation from the sun can kill pathogens in drinking water by three different mechanisms.

When UV, particularly in the germicidal range around 254 nm, is absorbed by DNA, adjacent thymine bases on a DNA strand bond covalently to form a thymine dimer. This dimer blocks protein synthesis and can terminate the replication of the DNA helix during the cell division cycle (Blake *et al.*, 1999). Also, incorrect pairs of thymine dimers can cause mutations (Oates *et al.*, 2003). It has been written that the disinfection capabilities of light decrease exponentially with the increase of wavelength from shorter UV range up to visible and infrared ranges (Acra *et al.*, 1987). It has been reported that UV and temperature have a synergistic effect in terms of disinfection at temperatures greater than 45°C (McLoughlin *et al.*, 2007).

Secondly, UV light can be absorbed by dissolved inorganic matter, inducing photochemical reactions that create highly reactive species. These include hydrogen peroxide, hydroxyl radicals and superoxide radicals, which are thought to lead to microorganism death by oxidising cellular components.

The third mechanism is the thermal effect of sunlight. Heat is created when water absorbs red and infrared light. At temperatures greater than the maximum growth value for a microorganism, denaturation occurs, impeding protein function (Oates *et al.*, 2003).

UV-A light is thought to have a sub-lethal, as well as lethal, effect on bacteria. This can mean that after they have received certain doses they: (i) do not grow on selective media but can be recovered on special catalase- and pyruvate-supplemented media; or (ii) display a lag time before continuing to grow (Berney *et al.*, 2007). In 1988, it was proposed that this growth delay could signify an adaptive response by the bacteria to UV-A light (Kramer *et al.*, 1988).

However, more recently a question has appeared over the viability of photolytic treatment alone as a method for disinfection, as bacteria regrowth has been reported (Rincón & Pulgarin, 2004a). Post-irradiation activity is discussed in detail in Section 2.5.5.

2.4.2.1 DNA and RNA

The nucleic acid contained within cells is the basic building block for all organisms and can be either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). DNA and RNA are long polymers made up of combinations of four nucleotides, adenine and guanine (purines) and thymine (this is replaced by uracil in RNA) and cytosine (pyrimidines). In the majority of cells, the nucleic acid constituent comprises of double-stranded DNA. In this case, nucleotides on one strand form hydrogen bonds with those on the other – adenine pairs with thymine (or uracil in the case of RNA) and guanine with cytosine.

2.4.2.2 UV-induced damage to nucleic acids

UV damages the nucleic acid within an organism, thereby disrupting cell replication. Nucleotides absorb light in the region of 200 – 300 nm, with pyrimidines being more susceptible to damage than purines (Jagger, 1967). The most common type of damage caused is the creation of a thymine-thymine dimer, a covalent bond between two thymine bases on the same DNA strand. Thus, it has been reported that organisms with high thymine content are more susceptible to UV-induced damage (Adler, 1966). Dimers cause disruptions in the transcription of information from DNA to RNA, interfering with a cell's metabolism. As will be seen in Section 2.4.2.3, this does not necessarily inactivate a cell, as it can respond by going photoreactivation or dark repair, whereby enzymes break the dimer (Blake *et al.*, 1999)

2.4.2.3 Repair mechanisms

Many microorganisms have developed repair mechanisms to reverse the effects of UV treatment. These fall into two basic categories, photoreactivation and dark repair.

Photoreactivation

Photolyase is an enzyme which repairs DNA sections that have been damaged by exposure to light. This repair is known as photoreactivation and takes the form of a two-step process. First of all, the photolyase recognises pyrimidine dimers and binds to them, forming an enzyme-dimer complex. The first step takes place independent of light. In the second step, however, light in the wavelength between 310 and 490 nm is used to break the dimer. Once this has happened, the photolyase becomes unbound. It is thought that photoreactivation may not always be sufficient for the microorganism to regain its infectivity, as shown in the case of *Cryptosporidium parvum* (Asano *et al.*, 2007). Not all bacteria possess photorepair mechanisms, *Micrococcus radiodurans* being one such example. Viruses do not contain the photolyase enzyme, but can obtain it from their host cell. It was thought that the probability of photoreactivation was inversely proportional to the applied dosage (Yip & Konasewich, 1972) and that the phenomenon was thus not of any practical significance in UV treatment plants (Rubbo & Gardner, 1965), but more recently some species such as *Legionella pneumophila* exhibited high photoreactivation capabilities after exposure to low- and medium-pressure light (Oguma *et al.*, 2004).

Dark Repair

Light-independent repair is achieved by three different mechanisms: excision repair, recombination repair and SOS repair.

Excision repair

Excision repair involves the sequential action of four enzymes. An endonuclease makes an incision at a damaged base; an exonuclease removes the damaged base; DNA polymerase fills the gap left during the removal; and DNA ligase forms the final bond after DNA synthesis by polymerase.

Recombination repair

This mechanism involves the replacement of a section of damaged DNA with a complimentary strand from another DNA molecule.

SOS repair

SOS repair is not yet fully understood, but it is defined as a bypass system which allows DNA chain growth across damaged segments. It is an error-prone system whereby incorrect bases can be formed and fidelity of replication is lost. SOS repair is the main cause of mutagenesis by UV light.

2.4.3 Studies on the removal of microorganisms by solar disinfection

A review of the effectiveness of solar disinfection was carried out by Gill & McLoughlin (2005), who took the results from a number of papers and calculated the comparative ease of disinfection of different microorganisms with respect to cumulative UV dose. As *E. coli* is the most widely used microorganism in solar disinfection studies, it was held as the reference point, as can be seen in Figure 2.13. The authors found that other Gram-negative showed similar disinfection kinetics to *E. coli*, although *Vibrio cholerae* was more resistant. Gram-positive bacteria were shown to be more resistant to disinfection than *E. coli*, which is not the case with many disinfection methods (see section 2.2.1.2). It can also be seen from Figure 2.13 that protozoa, fungi and viruses all tend to require higher doses of sunlight for disinfection to occur.

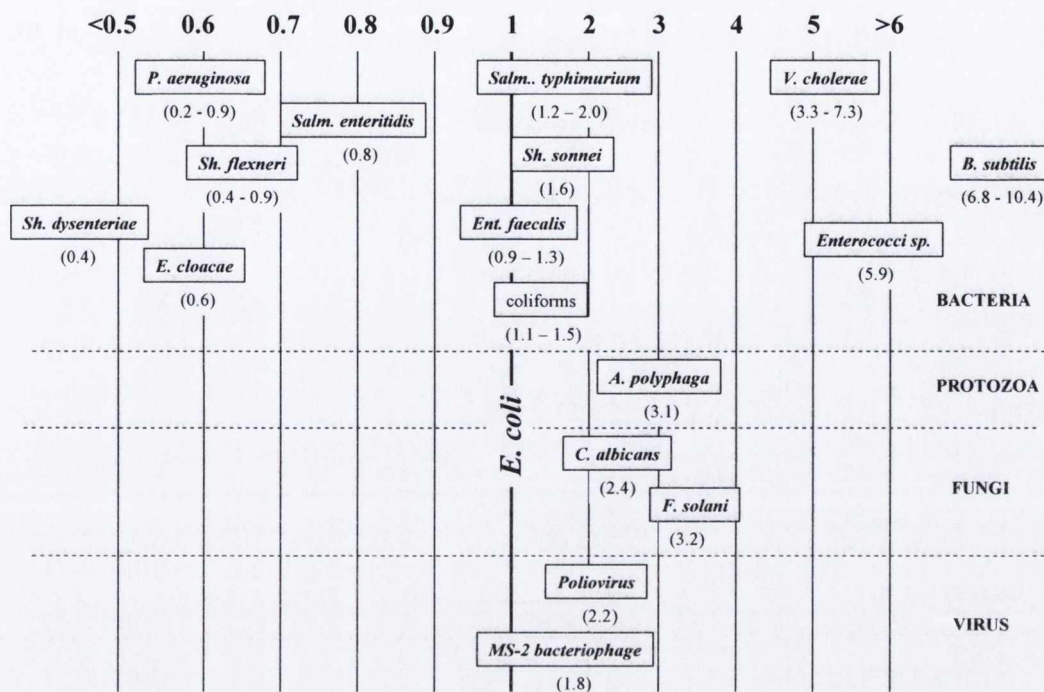


Figure 2.13. Relative solar disinfection resistance of various microorganisms in comparison to *E. coli* (Gill & McLoughlin, 2005)

2.5. Photocatalytic disinfection

2.5.1 The photocatalytic process: removal of organic pollutants

Photocatalysis was originally seen as an efficient method for the removal of chemical pollutants and only later was its disinfection potential investigated.

When a photon with energy greater than the bandgap energy (3.2 eV) is absorbed by a particle of TiO_2 , an electron is ejected from the valence band and jumps up to the conduction band. This leaves a hole in the valence band and an electron/hole pair is thus created (Figure 2.14). The holes are powerful oxidants, while the electrons are powerful reductants. Many of these electron/hole pairs recombine immediately, especially those deep within the TiO_2 molecule. However, if reactant species are present at the surface, oxidation and reduction reactions can occur. In an aqueous system, water acts a donor molecule, reacting with the holes to form the extremely reactive hydroxyl radical. The acceptor species is usually oxygen, which gains an

electron to form the superoxide radical. These radicals react with pollutants and lead to mineralization.

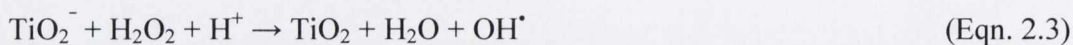
The mode of action of photocatalysis, as set out by Blake *et al.* (1999)

Electron-Hole Pair Formation

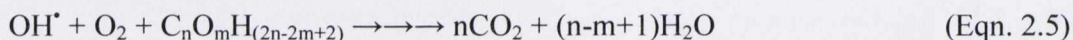


(conduction band electron and valence band hole)

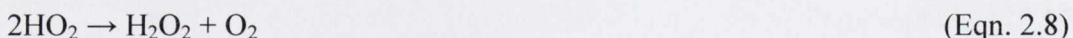
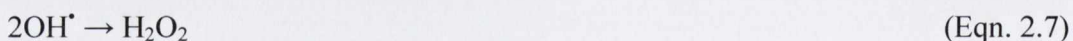
Electron removal from the conduction band



Oxidation of Organic Compounds



Nonproductive Radical Reactions



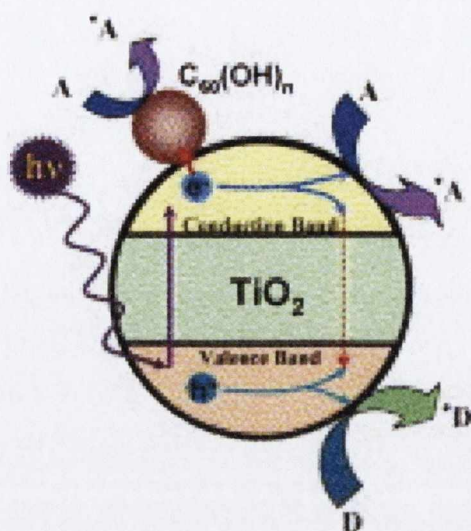
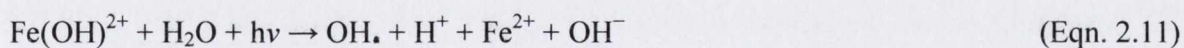


Figure 2.14 Schematic of electron – hole pair formation and subsequent oxidation/reduction reactions (Krishna *et al.*, 2006)

TiO₂ photocatalysis has been shown to be effective against a wide range of chemicals, such as azo-dyes (Augugliaro *et al.*, 2002), oxalic acid (Bandala *et al.*, 2004), gallic acid (Gumy *et al.*, 2006) and pesticides (Malato *et al.*, 2002).

2.5.1.1 Photo-Fenton Reaction

Degradation can also be achieved in the presence of an iron catalyst and hydrogen peroxide, via the photo-Fenton reaction. This process generates hydroxyl radicals according to the below equation, and these oxidise organic pollutants as above.



The photo-Fenton process's major advantage is that it can operate at wavelengths up to 600 nm, compared to 387 nm in the case of TiO₂ photocatalysis (Rincón & Pulgarín, 2007). This means that 35 % of solar irradiation is useful for the process (Moncayo-Lasso *et al.*, 2008b). It does, however, require a pH value in a limited range – from 2.5 to 4.0, due to the range of solubility of Fe-ions (Moncayo-Lasso *et al.*, 2008a). Also, homogeneous photo-Fenton treatment requires the recovery of Fe-ions after treatment to comply with drinking water regulations (Moncayo-Lasso *et al.*, 2008a). More recently, a study has shown that the photo-

Fenton process can be effective at near-neutral pH and at low temperatures and leads to both disinfection and the destruction of precursors of disinfection byproducts (Spuhler *et al.*, 2010).

2.5.2 Mode of killing of cells via photocatalysis

The disinfection of water using TiO₂ has been under investigation since Matsunaga *et al.* reported the complete sterilisation of *Lactobacillus coli* when subjected to UV irradiation in the presence of platinum-loaded TiO₂ (Matsunaga *et al.*, 1985). There is still some speculation as to the exact pathway of cell attack, but it has been proposed that oxidative damage takes place where the TiO₂ surface comes into contact with the cell wall. The cytoplasmic membrane then comes under attack once the cell wall has been sufficiently damaged and the cell permeability is increased until the free efflux of intracellular components is permitted. This eventually leads to cell death (Huang *et al.*, 1999). This explanation was echoed by Sunada *et al.* (2003) who studied cell morphology during photocatalytic treatment of *E. coli*. They set out the process by which cells die due to photocatalytic treatment as follows: The outer membrane is partially decomposed by the reactive species created during TiO₂ photocatalysis. This in itself does not have a major impact on cell validity but makes the cell more permeable to reactive species. Thus, the cytoplasmic membrane is attacked by the reactive species and this leads to the lipid peroxidation of the membrane (Sunada *et al.*, 2003). When the membrane is damaged beyond repair, cell respiration ceases (Dunlop *et al.*, 2002). The theory set out by Sunada *et al.* has been backed up by other authors, who, during the photocatalytic treatment of *E. coli* using TiO₂, observed the production of MDA (malondialdehyde), which indicates peroxidation of the membrane lipid (Maness *et al.*, 1999). Similarly, in experiments carried out on *Streptococcus sobrinus*, Saito *et al.* (1992) concluded that the reactive oxygen species damage the cell membrane and the increased permeability allows RNA, protein and potassium to leak out, causing cell death. The destruction of the cell wall was not deemed essential for loss of cell viability but was seen as a secondary phenomenon. They deduced that radicals were responsible from the fact that cell death occurred within a minute, whereas TiO₂ particles took more than 30 minutes to reach the membrane after the peptidoglycan layer had been decomposed (Saito *et al.*, 1992). This was strengthened further by research that showed a linear correlation between the production of hydroxyl radicals and the disinfection of *E. coli* (Cho *et al.*, 2004). SEM and ¹⁴C radioisotope experiments were also used to show that hydroxyl radicals are extremely influential in cell death (Jacoby *et al.*, 1998). Salih (2002), however, argued that the hydroxyl radical might not contribute directly to cell death. He

calculated from the diffusion coefficient and lifetime that the radical would not travel far enough to pass through the cell membrane. This would mean there would be no direct attack on nuclear components, which are responsible for about 90% of radiation-induced death (Munro, 1970). He cited Saito's theory as a possible explanation of the process.

Another process by which cell inactivation can occur is a naturally occurring Fenton reaction. Iron occurs within cells as proteins and on the surface of cells and provides a source of ferrous ions. The combination of these and the hydrogen peroxide produced by TiO₂ photocatalysis allows the photo-Fenton reaction to proceed, thus generating hydroxyl radicals. This process can continue in the dark via the Fenton reaction.

In summary, the general consensus with regards to the photocatalytic cell killing mechanism is that the oxidative radicals produced target the cell wall, damaging the lipopolysaccharide layer and the peptidoglycan layer. Then lipid peroxidation of the membrane occurs, along with the oxidation of polysaccharides and proteins.

2.5.2.1 Bacterial adsorption

One of the as yet unanswered questions surrounding photocatalytic disinfection is whether bacterial adsorption to the photocatalyst is necessary for disinfection to take place. A few authors have mentioned that the hydroxyl radical is short-lived (Blake, 1999, Salih, 2002, Frazer, 2001) and would not diffuse from the TiO₂ surface, its lifetime being measured as 8.7 ns (Roots and Okada, 1975). If it is to have a bearing on the destruction of the cell wall and membrane, therefore, attachment would be necessary. *Ct* values (a concentration-time expression of minimum dose) for hydroxyl radicals have been shown to be 10²–10⁶ times lower than ozone, chlorine and chlorine dioxide, meaning very small concentrations of hydroxyl radicals in effect for small periods of time could be effective (Cho *et al.*, 2003). In fact, due to the size of TiO₂ particles, they can penetrate into the target cells. Guillard *et al.* (2007) used a dialysis membrane to investigate the importance of the contact between bacterial cells and photocatalyst particles. The membrane was used to envelope a small suspension of *E. coli*, with the P-25 TiO₂ being kept outside of the envelope. After five hours of irradiation, no inactivation of bacteria occurred, leading the authors to two conclusions: Firstly, that hydrogen peroxide is not an important contributor to the inactivation of bacteria

during photocatalysis and, secondly, that a contact or very close proximity between bacteria and irradiated photocatalyst is required.

2.5.3 The differences between the photocatalytic removal of organic pollutants and photocatalytic disinfection

The main differences between photocatalytic disinfection characteristics of organic pollutants and microorganisms were outlined as follows (Guillard *et al.*, 2008):

2.5.3.1 Size

Organic molecules, such as pesticides, dyes and VOCs tend to be below 1nm in size, whereas microorganisms are usually greater than 300 nm. This means that photocatalyst particles (of typical size 30 nm) are small enough to wrap and penetrate into cells. As such, the Langmuir adsorption seen in the case of organic pollutants is not followed by microorganisms. The response to photocatalytic treatment is obviously different, as many microorganisms have repair mechanisms and are able to adapt to change. As living things, the viability of bacteria varies dramatically depending on the stage of growth (Rincón and Pulgarín, 2004), although this variation has been shown to be species-specific. Reasons for this have been suggested as the presence of different levels of SOD and CAT and the ratio of unsaturated to saturated fatty acids in cells (Leung *et al.*, 2008).

2.5.3.2 Composition

As discussed in Section 2.2, microorganisms have very varied and complex make-ups. Even among bacteria the Gram-negative bacterial cell has multiple layers of protection, with the slime layer, cell wall and cell membrane all providing significant resistance to external species, whereas Gram-positive cells have a far thinner cell wall and tend to be more prone to disinfection techniques.

2.5.3.3 Viability

Many organisms possess repair mechanisms which mean that even after damage has occurred, cells can recover, differentiating them from chemical compounds. In the case of bacteria, these repair mechanisms are outlined in Section 2.4.2.2.

2.5.3.4 Inactivation process

It is difficult to compare chemical and biological pollutants, as the efficiency of the photocatalytic process is measured in different ways for either case. With organic compounds, the mineralization of the molecules indicates effective photocatalysis. On the other hand, microorganisms go through a complicated series of events until total inactivation is achieved.

2.5.4 Photocatalytic experiments undertaken against different organisms

2.5.4.1 Bacteria

E. coli has been used as a target organism in the vast majority of experiments looking into photocatalytic disinfection (Benabbou et al., 2007, Dunlop et al., 2001, Ireland et al., 1993, Matsunaga & Okochi, 1995, Salih, 2002, Sun et al., 2002, Sunada et al., 2002, Wei et al., 1994). However, the inactivation of other bacteria has been investigated using TiO_2 as a photocatalyst, for example total kill was achieved of *Staphylococcus aureus* and *Serratia marcescens* in just 10 and 8 minutes respectively, compared with 7 minutes for *E. coli* under the same conditions (Block et al., 1997). In this paper, total kill for *Serratia marcescens* was not achieved after 60 minutes when there was no photocatalyst present. The Gram-negative bacteria *Salmonella typhimurium*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* were all found to be inactivated by photocatalytic treatment, which is significant as *E. cloacae* cannot be killed by photolytic treatment alone (Ibanez et al., 2003). Another study showed that sunlight had strong disinfective capabilities when *Aeromonas hydrophila* and *Pseudomonas fluorescens* were used as test organisms and interestingly the deactivation of the bacteria was not influenced by the addition of TiO_2 . Indeed, this prompted the authors to proffer the rather bold statement that the observations ‘indicate that the photocatalytic approach does not offer real prospects as an alternative technology for the disinfection of drinking water’ (Kerstens et

al., 1998). Other bacteria successfully treated using TiO₂ photocatalysis include *Streptococcus sobrinus* (again, photolysis alone was shown to be completely ineffective in the same time frame required for complete disinfection by photocatalysis) (Saito *et al.*, 1992) and *Enterococcus faecium* (Kühn *et al.*, 2003). Caution must be taken when comparing these tests, as a number of different variables will influence the experimental results, such as whether natural or artificial light was used, whether the TiO₂ was supported or suspended, the reactor type and size and the intensities used.

2.5.4.2 Viruses

Less research has been conducted on the inactivation of viruses, but *Poliovirus 1* (Watts *et al.*, 1995), *phage MS2* (Sjogren & Sierka, 1994), and *B. fragilis bacteriophage* (Laot *et al.*, 1999) have all been tested. *Poliovirus 1* inactivation rate kinetics were shown to follow Chick's Law (Eqn. 4.1) and the virus was inactivated more quickly than *E. coli* under the same conditions.

2.5.4.3 Fungi

Candida albicans and *Fusarium solani* were completely inactivated by both SODIS and SPC-DIS, with the inactivation rate being much quicker when the photocatalyst was present (Lonnen *et al.*, 2005). Five wild strains of the *Fusarium* genus (*F. equiseti*, *F. oxysporum*, *F. anthophilum*, *F. verticillioides*, and *F. solani*) were treated using both solar photocatalysis and solar photolysis. Again, complete disinfection was achieved by both methods but a rate enhancement was witnessed when a photocatalyst was used (Sichel *et al.*, 2007).

2.5.4.4 Protozoa

The inactivation of *Clostridium perfringens* spores was found to be directly proportional to incident light intensity in photocatalytic experiments (Dunlop *et al.*, 2007). The viability of *Cryptosporidium parvum* oocysts was reduced from 98.3 % to 11.7 % after 16 h of treatment with a photocatalyst in overcast conditions. Without TiO₂, the viability only fell to 36.0 % under the same conditions (Méndez-Hermida *et al.*, 2007). It was found that neither solar nor solar photocatalytic batch reactors were effective against the cysts of *Acanthamoeba polyphaga* (Lonnen *et al.*, 2005).

2.5.5 Post-Irradiation Bacterial Activity

One of the greatest advantages photocatalysis is thought to have over photolysis is the prevention of post-irradiation survival of bacteria (Rincón & Pulgarín, 2004a). Killing or removing the microorganism responsible for a pathogenic or allergic response is often not enough as some bacteria produce endo- and exotoxins (Blake *et al.*, 1999), contact with which can give rise to medical problems (Morey *et al.*, 1990). It is a cause for concern that cell structures which persist even after the cell is no longer viable can give rise to allergic responses. It has been found that bacteria have evolved four main repair mechanisms in the repair or damage tolerance of UV radiation-damaged DNA, including photoreactivation, nucleotide excision repair (NER), mutagenic (or SOS) DNA repair (MDR), and recombinational DNA repair (Friedberg *et al.*, 1995). These were outlined in Section 2.4.2.2.

It is also reported that all aerobic organisms have developed a superoxide dismutase (SOD) enzyme to dismutate O_2^- to H_2O_2 and O_2 , and a catalase enzyme to convert H_2O_2 to H_2O and additional O_2 as innocuous end products. This counters the effect of superoxide radicals and hydrogen peroxide.

As well as this, several prokaryotes including *E. coli* have evolved to produce different forms of SOD strategically distributed within a cell to shield the cells from any oxidative damage resulting from their normal metabolism.

In *E. coli*:

- Fe-SOD along the periphery of the cytoplasm close to the inner membrane to dissipate radicals produced from aerobic respiratory pathways.
- Mn-SOD is more abundant in the central region of the cell where the nucleoid is in order to protect DNA from damage caused by oxidants.
- Cu,Zn-SOD, exclusively in periplasm, is meant to guard cells from any exogenous source of O_2^- produced from immediate hostile environment or by other phagocytic cells. (Blake *et al.*, 1999)

From previous experiments it has been inferred that photocatalytic treatment has a 'residual' bactericidal effect, whereby decay in bacterial concentrations continues to occur after treatment has stopped. A comprehensive list of hypotheses as to why a residual effect is seen

in such experiments has been presented. These include the fact that some enzymes responsible for the defence of bacteria against oxidative stress may continue in the dark; the altered membrane permeability may allow chemicals to enter into the cells, meaning the perturbation of redox reactions in the cell may continue in the dark; and the other by-products of photocatalysis may continue to attack cells in the dark (Rincón & Pulgarín, 2007).

It has been found that damage caused to cells can continue in the dark via the free radical chain reactions of lipid peroxidation, once lethal oxidation reactions have been initiated by TiO_2 photocatalysis (Huang *et al.*, 2000). This shows a clear advantage of photocatalysis over photolytic disinfection, as complete termination of cells is achieved when the treated water is stored for 24 hours because of post-irradiation die-off. This has encouraging implications, as it shows that potentially harmful bacteria regrowth can be prevented in the presence of a photocatalyst.

The same authors also found that regrowth/reactivation of bacteria occurred after photolytic treatment of bacteria, even when a bacterial concentration < 1 CFU/mL had been obtained (Rincón & Pulgarín, 2004a). However, to verify the complete inactivation of *E. coli* in the presence of suspended TiO_2 , post-treatment samples need to be incubated in ideal growth conditions. It is thought that after photolytic treatment, bacteria can reproduce in the dark or damaged bacteria can regrow, whereas photocatalytic treatment can continue to have an effect post-irradiation (Huang *et al.*, 2000). In fact, it was previously concluded from regrowth experiments that TiO_2 treatment has a bactericidal effect, causing irreversible damage in cellular functions, while solar treatment alone merely has a bacteriostatic effect (Gelover *et al.*, 2006). This is a particularly attractive characteristic for continuous flow reactor design where the disinfected water is likely to be stored in a closed reservoir for some time before consumption.

2.5.5.1 Effective Disinfection Time

Effective Disinfection Time (EDT) was developed as a 'measure of disinfection' and is defined as the treatment time necessary to avoid some bacterial regrowth after 24 (or 48) hours in the dark, after irradiation had stopped (Rincón & Pulgarín, 2004b). It depends on parameters such as the type of bacteria and the initial bacteria concentration. In early

experiments comparing photolytic and photocatalytic systems, the EDT was not reached in photolytic studies even if after illumination no bacteria were detected. In contrast, the EDT was achieved after 60 minutes of illumination in the presence of a TiO_2 photocatalyst (Rincón & Pulgarín, 2004b).

2.5.6 Parameters affecting photocatalytic disinfection

2.5.6.1 Turbidity

As outlined by Rincón and Pulgarín (2003), there are a number of reasons why turbidity has a negative effect on photocatalytic disinfection of water.

- a) Bacterial growth is enhanced by the adsorption of nutrients on particle surfaces, giving the attached bacteria more favourable growth conditions. Bacteria attached to particulate matter are also shaded from the incident light more than those in suspension.
- b) Turbid water is generally warmer, as particles absorb heat from the sunlight. Warmer water contains less oxygen, meaning cell respiration is more difficult and fewer $\bullet\text{OH}$ radicals are produced.
- c) Suspended organics compete with the bacteria for adsorption sites on photocatalyst particles and for $\bullet\text{OH}$ radicals.
- d) Suspended particles scatter the incident sunlight, thus reducing solar penetration.
- e) The efficiency of TiO_2 particles is reduced by their aggregation to the suspended particles.

2.5.6.2 pH

Studies have found that the pH of water undergoing photocatalytic treatment in the presence of TiO_2 drops significantly during the process. This is partly due to the production of H^+ ions when $\bullet\text{OH}$ radicals are formed. Experiments were conducted in which the pH drop was simulated using hydrochloric acid in the absence of TiO_2 , with the result that the disinfection rate was much higher in this case than during a photolytic test with no addition of acid, during

which the pH remained at 7. A pH decrease in the dark did not have a significant effect on disinfection (Rincón & Pulgarín, 2004).

Watts *et al.* (1994) found that the inactivation rate for poliovirus 1 and coliform bacteria was unaffected by varying the initial pH from 5 to 8. Similar results were found by other authors, who observed very little change in inactivation rate of *E. coli* when the initial pH was varied from 4 to 9. The inactivation rate of *E. coli* was slightly higher at an initial pH of 4 or 9 than it was at 7, due to the sensibility of bacteria to extreme pH environments and to the fact that TiO_2 agglomeration states are a function of pH. The authors also suggest that the fact that acid-adapted cells can increase tolerance to osmotic stress explains why varying the initial pH has very little influence on photocatalysis (Rincón & Pulgarín, 2004).

2.5.6.3 Dissolved Oxygen

As Reactive Oxygen Species (ROS) are widely believed to be the cause of cell death during photocatalysis, the dissolved oxygen content of the water undergoing treatment is vital. At low levels of DO, the electron-hole pairs simply recombine. Oxygen molecules do not compete with contaminant molecules for adsorption sites on the surface of TiO_2 , as oxidation and reduction reactions do not occur at the same sites (Gerischer, 1992, Pelizzetti, 1990). Authors have observed a steep decline in dissolved oxygen concentration during photocatalytic disinfection experiments (Rincón and Pulgarín, 2005), which can be explained by microorganism respiration but also by the following reactions during the photocatalytic process:



(Rincón and Pulgarin, 2005).

2.5.6.4 Temperature

Temperature is a very important parameter in solar disinfection, having a synergistic effect at temperatures above 45°C (Wegelin *et al.*, 1994). While temperature has no effect on the photocatalytic process between 20 °C and 80 °C (Malato *et al.*, 2009) raising the temperature during photocatalytic disinfection will obviously have a pasteurizing effect on microorganisms present in the water. Higher temperatures usually mean lower DO levels, which have a negative effect as discussed previously. It has been found that temperature affects different organisms in completely different ways. It was observed that the disinfection efficiency of Gram-positive bacteria was increased and that of Gram-negative bacteria (not including coliforms) decreased when temperature was raised from 23°C to 45°C (Rincón & Pulgarín, 2003). It was also found that photocatalytic disinfection of *A. alvinellae* and *P. phosphoreum* was enhanced greatly when the temperature was raised from 15°C to 37°C (Leung *et al.*, 2008)

2.5.6.5 The presence of organics

The influence of organics is of some concern. During most laboratory experiments looking into photocatalytic disinfection, sterile or deionised water is usually used. This, however, does not give a true representation of real conditions. Authors have found that organics can either aid photocatalysis by enhancing ROS production or hinder it by competing with organisms for oxidative species (Rincón & Pulgarín, 2004).

2.5.6.6 The presence of inorganics

Ions such as chlorine, phosphate and sodium can inhibit photocatalytic processes as they are likely to adsorb onto TiO₂ surfaces and compete with target organisms for reactive species (Robert & Malato, 2001, Rincón & Pulgarín, 2004). Phosphate is thought to be the most disruptive of the salts (Blake *et al.*, 1999) and potassium ions were, for example, proven to retard the photocatalytic process more so than sodium ions (Rincón & Pulgarín, 2004).

2.5.6.7 Light Intensity

Unsurprisingly, photocatalytic disinfection efficiency is known to increase as the intensity of incident radiation increases. This has been demonstrated in photocatalytic experiments, where it was found that increased UVA light intensity brought about higher inactivation rates (Benabbou *et al.*, 2007, Rincón & Pulgarín, 2004). An interesting study was conducted into the effect of intermittent light intensity, from which it was deduced that different organisms react in different ways to this phenomenon and that the time of interruption is critical. If irradiation is stopped near the beginning of the process, it is much more detrimental than the same interruption after a long period of illumination (Rincón & Pulgarín, 2003).

2.5.6.8 Wavelength of light

An experiment was conducted to investigate the effect of UV-A, UV-B and UV-C light on the photocatalytic process (Benabbou *et al.*, 2007). Total inactivation of *E. coli* was observed after 90 min, 60 min and 20 min for UV-A, UV-B and UV-C, respectively. The initial inactivation trends seen in the cases of UV-A and UV-B were similar, but the authors attribute the comparative drop in efficiency of UV-A to autorepair and self-defence mechanisms. UV-C photolysis was shown to be more effective than UV-C photocatalysis, meaning that photocatalysis is of more interest when solar energy is used.

2.5.6.9 Initial photocatalyst concentration

The photocatalytic efficiency of systems tends to increase with increased photocatalyst concentration up to a certain value, which has been found to be in the region of 1.0 g/L for many systems (Wei *et al.*, 1994, Li *et al.*, 1996, Maness *et al.*, 1999). At higher concentrations, it is thought that the increased turbidity is detrimental to the process as photocatalyst particles shade each other, and target organisms, from incident light. In one paper, the optimal concentration determined from experiments (0.25 g/L) was compared with a mathematical estimation of the concentration of TiO₂ required to completely coat each bacterial cell, but this did not yield a good correlation (Benabbou *et al.*, 2007).

2.5.6.10 Concentration of target organisms

With a higher initial cell loading, the probability of contact between cells and photocatalyst particles is higher. This leads to a higher disinfection rate, a result which has been observed by many authors (Dunlop *et al.*, 2002, Pham *et al.*, 1995).

2.6. Photocatalysts

2.6.1 Titanium dioxide

Photocatalysts which have been used in the degradation of chemical and biological pollutants include CdS (Maruska & Ghosh, 1978), ZnO (Augugliaro *et al.*, 1988), In₂O₃ (Sato, 1988), ZnS and Al₂O₃ (Karunakaran & Anilkumar, 2008). It has been repeatedly found, however, that titanium dioxide, TiO₂, is the most effective and gives the greatest quantum yield (Herrmann, 1999).

The advantages of using TiO₂ as a photocatalyst have been highlighted by many authors:

- It is affordable and readily available
- Systems using TiO₂ as a catalyst require little maintenance and can operate unattended
- It requires no liquid chemicals
- It operates at ambient pressure
- Systems can be easily adapted to suit local needs
- It is known to degrade a wide range of chemical and biological pollutants
- It is not known to produce hazardous byproducts

(Pichat *et al.*, 2004; Sano *et al.*, 2004; Block *et al.*, 1997 ; Herrmann, 1999 ; Rincón & Pulgarin, 2004)

Titanium is the ninth most abundant element on the planet (Donachie, 2000), known for its high strength and corrosion resistance. Titanium dioxide can take the form of one of three crystal structures rutile, anatase and brookite (Figure 2.15). Rutile is the most stable form

(Hatch, 1949), while anatase is most often used for photocatalytic purposes due its higher activity (Aegerter & Mennig, 2004). Also, the formation of anatase is favoured kinetically at temperatures less than 600°C, which is thought to lead to its having a greater surface area and greater number of active sites for adsorption and catalysis (Herrmann, 1999). The Fermi level of anatase is about 0.1 eV higher than that of the rutile phase, meaning anatase has a lower oxygen adsorption capacity and thus a higher level of hydroxylation (Bannerjee *et al.*, 2006). The type of TiO₂ used is important in photocatalytic oxidation and disinfection experiments, as the formation of radical species is dependent on parameters such as particle size, crystalline phase, uniformity, BET area, etc. (Gumy *et al.*, 2006). Blends of rutile and anatase are available commercially, such as the widely used Degussa P25 (80% anatase, 20% rutile), which has been found to be more effective at the photocatalytic inactivation of *E. coli* than a pure anatase form (Rincón & Pulgarín, 2003). In the same paper, the authors found rutile to be more effective than anatase and suggested, among others, the following reasons: anatase reflects more UV light than rutile, meaning that less is available to bacteria; rutile has a higher refractive index, allowing more light to penetrate the bacterial suspension; and rutile absorbs more strongly near ultraviolet wavelengths. The authors concede, however, that rutile has a higher relative opacity than anatase, which limits light penetration somewhat. The reason for the different results found when comparing anatase and rutile is not fully understood and further experiments have been recommended to find out why this may be so, although explanations such as crystal size, porosity, defects and pore size distribution have been proffered for the disagreement of the results (Bannerjee *et al.*, 2006). Sato & Taya (2006) investigated the influence of TiO₂ crystalline structure on the photocatalytic inactivation of phages and discovered that a combination of rutile and anatase, in the ratio 30:70, was more efficient than either on its own. They hypothesized that this was due to the balanced acceleration of oxidation and reduction rates, as ROS generation in anatase is oxidation limited and in rutile is reduction limited (Figure 2.16).

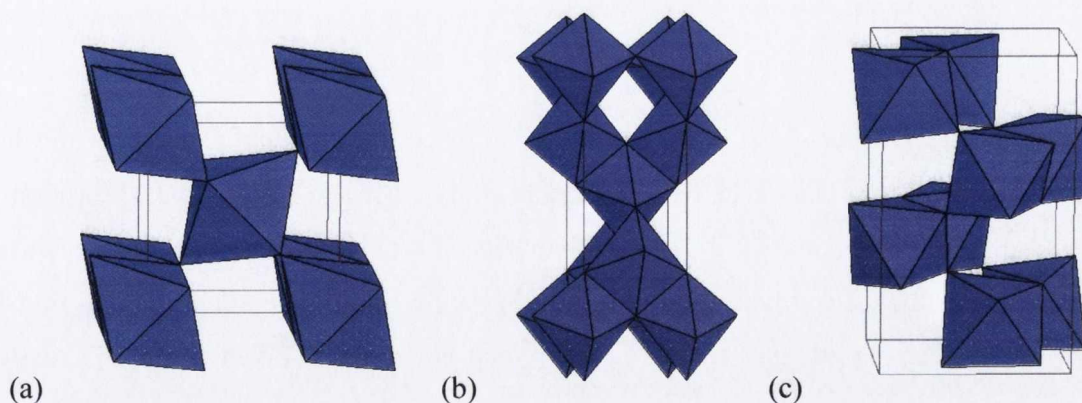


Figure 2.15 The crystal structures of (a) rutile, (b) anatase and (c) brookite (ruby.colorado.edu/~smyth/min/tio2.html)

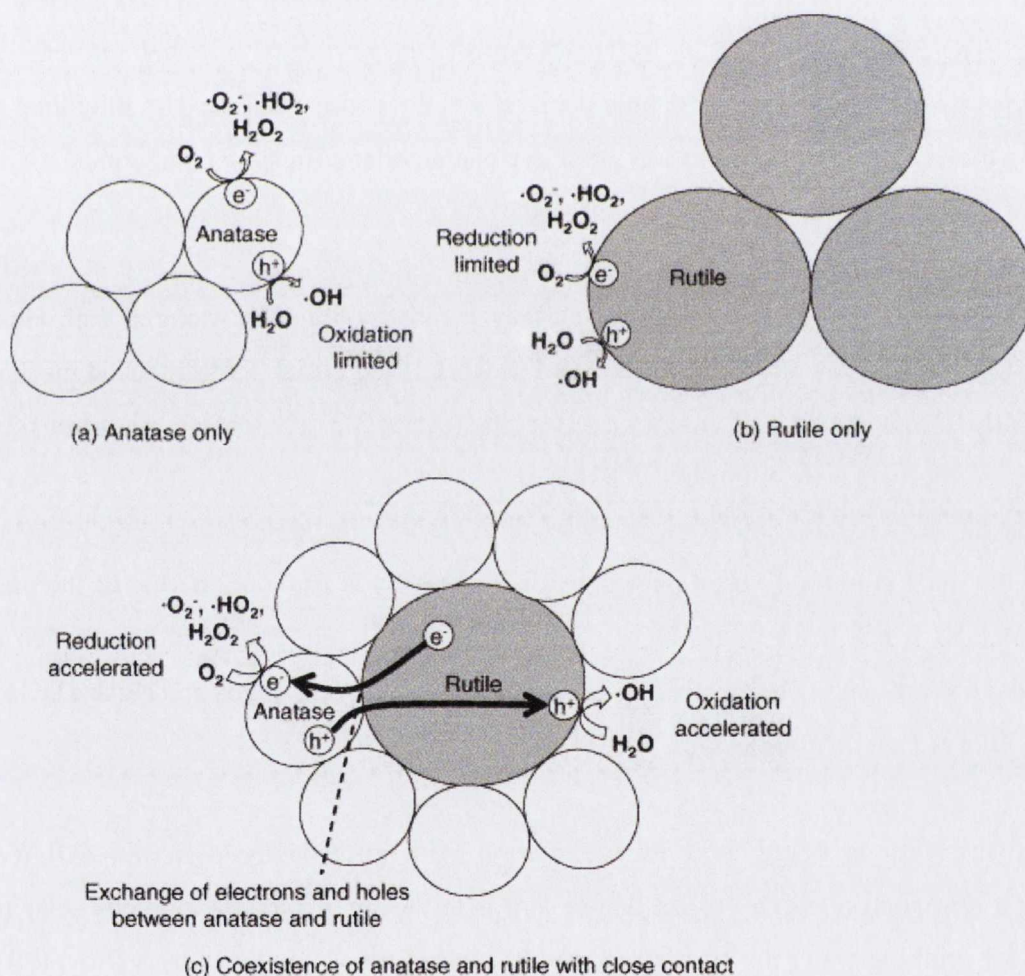


Figure 2.16 Possible mechanism for enhancement of photocatalytic activity caused by contact between anatase- and rutile-types of TiO_2 (Sato & Taya, 2006)

2.6.2 Photosensitisers

Photosensitisation (Figure 2.17) is defined as the process by which a photochemical or photophysical alteration occurs in one molecular entity as a result of initial absorption of radiation by another molecular entity called a photosensitizer (IUPAC, 1997). Photosensitisers are most often coloured compounds which absorb visible light, instigating electron transfer reactions or transferring the light energy direct to their environment (Wainwright & Crossley, 2004).

Photosensitization is useful in the photocatalytic process as it can produce singlet oxygen, a highly reactive species which has the ability to attack proteins, lipids and nucleic acids, causing irreversible damage in cells (Navntoft *et al.*, 2007, Sichel *et al.*, 2005). Photosensitisers such as methylene blue deraivatives have also been used as microbial stains, meaning they have high selectivity, an attractive characteristic for such compounds.

It has been found that cationic photosensitisers are generally more effective at inactivating Gram-negative bacteria, such as *E. coli*, as they localise within the bacterial cell. However, anionic photosensitisers have also been found to work if the initial photo-induced mechanisms sufficiently damage the cell membrane for the extracellularly-located photosensitiser to relocate internally (Lacey & Philips, 2001).

One of the most commonly used photosensitiser elements is Ruthenium, one of the platinum group metals. It has been found to be highly effective at sensitising singlet oxygen (Juris, 1988) and has proven to be effective against many different compounds and cells (Davia *et al.*, 2008, Mikel & Potvin, 2002).

Photosensitization is being used in conjunction with photocatalysis in the SOLWATER project, a European research project whose aim is to design a fully autonomous solar reactor system for drinking water purification in remote parts of developing countries (Navntoft *et al.*, 2007).

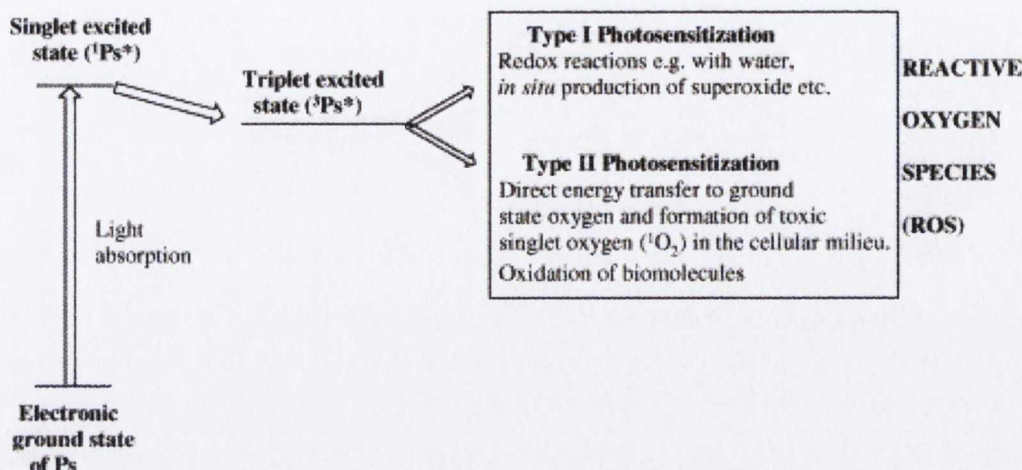


Figure 2.17 Photosensitisation pathways for a photosensitiser ‘Ps’ (Wainwright & Crossley, 2004)

2.6.3. Methods of fixing photocatalysts

Although reaction rates are lower when fixed photocatalysts are used, they prevent the need for expensive separation of suspended TiO_2 at the end of treatment. Fixed photocatalysts are less effective due to the lower catalyst surface area to volume ratio and decreased mass transfer. It has been found that when thin films are used, the thickness of the film and irradiated area are the main factors affecting reaction rate. If films are too thick, the electron hole pairs are located too deep within the film and thus cannot reach the surface in order to react within their lifetime. If a layer is too thin, again this can have a negative effect on disinfection, as only a small fraction of radiation would be absorbed (Vesely *et al.*, 2005). As there is no adsorption of bacteria on the fixed TiO_2 and that reactions occur certainly mainly at (or close to) the surface of TiO_2 and not in the solution, the average distance between bacteria and catalyst appears to be an important limiting factor for the photocatalytic inactivation of bacteria. The average half-distance between bacteria and immobilized TiO_2 increases and the probability of attack by $\cdot\text{OH}$ decreases as compared to suspended TiO_2 (Gumy *et al.*, 2006). Two main methods are used for fixing TiO_2 : (a) using a powder such as the Degussa P25 employed in the experiments carried out in this research; (b) the preparation of the catalyst *in situ* using a sol-gel method.

2.6.3.1 Powdered TiO₂

TiO₂ powder fixed on paper

TiO₂ fixed on paper is commercially available from companies such as Ahlstrom. The obvious disadvantage of this paper is its opacity to light, which significantly restricts its photocatalytic capabilities. This is the chosen support system used in the SOLWATER reactor, mentioned in Section 2.7.4.

Dip-coating of other supports

Dip-coating is achieved by dipping the support in a suspension of TiO₂ before drying and flushing the support. Many different materials have been investigated for use as supports in photocatalytic reactors. Glass has been looked into in the form of beads, tubing, fibres and plates (Bideau *et al.*, 1995), while polymers (Bellobono *et al.*, 1992), sand (Matthews, 1991) and ceramics (Kato, 1993) have also been considered.

Electrodes

It has been found that by applying a reverse bias to electrodes coated in TiO₂, bacterial cells are attracted to the photocatalyst surface, thus accelerating the rate of reaction (Dunlop *et al.*, 2002). It also ensures that fewer electron-hole pair recombination events take place, meaning there are more electrons and holes available for oxidation and reduction reactions.

2.6.3.2 The sol-gel technique

The great advantage of the sol-gel technique is that it leads to a fixed layer of photocatalyst which is transparent to light. It requires much higher temperatures than the more simple dip-coating technique but the resultant films adhere more tightly to the support surface and are not easily scraped off. In the sol-gel process, a titanium salt is converted to titanium hydroxide by hydrolysis and then to TiO₂ by pyrolysis. The initial salt is usually a hydroxide, as it is hard to control the hydrolysis of inorganic salts such as TiCl₄ in the liquid phase (Bideau *et al.*, 1995). Titanium tetraisopropoxide is the most commonly used salt for the sol-gel method. Other

advantages of the technique, as outlined by Tracey *et al.* (1998) include the possibility of changing the sol composition, thus producing a change in film microstructure; relatively low processing cost and temperature; and the problems of producing a good stoichiometric ratio of elements and molecular homogeneity are overcome (Tracey *et al.*, 1998). It is thought that while sol-gel methods are transparent to light, their smoother surface morphologies are less conducive to the killing of bacteria.

Spray Pyrolysis

Spray pyrolysis is a cheap and simple method for producing nano-crystalline films. and it has been found that their surface morphologies are specular (Diaz *et al.*, 2007). A schematic of the spray pyrolysis technique can be seen in Figure 2.18.

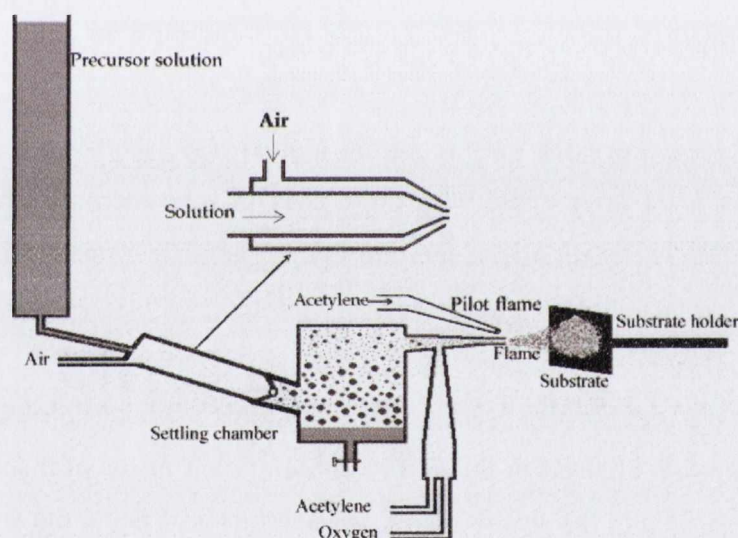


Figure 2.18 The spray pyrolysis technique (Kavitha et al, 2007)

Spray-gel

This technique is a combination of the spray pyrolysis and sol-gel techniques. It involves creating an aerosol from the sol and spraying this onto a heated substrate, where the film grows (Diaz *et al.*, 2007). It has been shown that this technique creates rougher films than the spray pyrolysis method, which show better photocatalytic behaviour (Quintana *et al.*, 2005). In

order to achieve a uniform coating of the reactor tubing, an axially-mounted medical nebuliser can be used (Diaz *et al.*, 2007).

2.6.4 Doping

The photocatalytic performance of fixed TiO₂ has been shown to improve with the addition of certain chemicals. It works by broadening the wavelength to which TiO₂ particles respond (Yu *et al.*, 2007). This would further open up the possibilities of solar photocatalysis as a viable treatment method. It has been found that nitrogen is an appropriate doping chemical for tuning the band gap of TiO₂ for absorption of the visible light spectrum (Yu *et al.*, 2007).

2.7. Reactor Design

2.7.1 Reactor Profiles

Despite photocatalytic processes only requiring high-energy, short-wavelength photons, the reactors used have a lot in common with those used in solar-thermal applications (Blanco-Galvez *et al.*, 2007). The main differences are that no thermal insulation is required and the absorber must be transparent to photons.

Solar collectors have traditionally been grouped into three main categories, depending on the degree of concentration attained in them. The concentration factor of a solar collector is the ratio of the aperture area to the absorber area, the aperture area being the area intercepting the radiation and absorber area the area receiving the radiation.

The three categories are:

- Non-concentrating (low concentration or low temperature) systems
- Medium-concentrating (medium temperature) systems
- High-concentrating (high temperature) systems

2.7.1.1 Non-concentrating systems

Non-concentrating systems are static systems which have no solar tracking mechanism. Thus, they are inclined towards the sun at the optimum angle, which depends on the latitude of the site in question. They are simple, cheap systems which can make use of both direct and diffuse radiation. An example is a flat-plate reactor, which can be seen in Figure 2.19. In photocatalysis, an advantage associated with non-concentrating systems is that they collect diffuse radiation [the collection of diffuse radiation is inversely proportional to the concentration factor (Rabl, 1985)], meaning more of the high energy photons required for photocatalysis are collected.

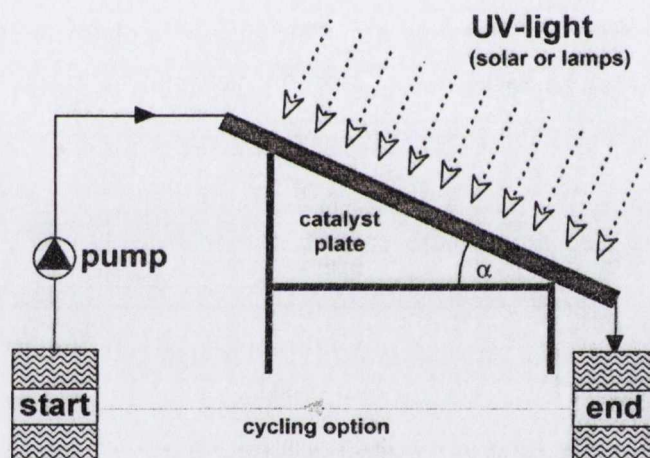


Figure 2.19 Flat-plate reactor system (<http://netserv.ipc.uni-linz.ac.at/~dieter/DsWeb/Research/Detox/FlachbSchema.jpg>)

2.7.1.2 Medium-concentrating systems

Medium-concentrating reactors, such as parabolic trough reactors (PTRs) and holographic collectors (Fresnel lenses), usually move about one or two axes to follow the path of the sun. PTRs were the first collectors to be used for solar photocatalytic applications due to the importance of this type of collector in solar thermal applications and because they were considered the most suitable of the concentrators available at the time (Blanco-Galvez *et al.*, 2007). A PTR concentrates the light on its parabolic surface, reflecting it onto a tubular

receiver in the focus (Sichel *et al.*, 2005). PTRs concentrate the direct rays of the photocatalytically active UV part of the solar spectrum by a factor of 20–50 (Alfano *et al.*, 2000). Holographic reactors employ lenses which refract incoming light onto a linear focus (Gupta and Traugott, 1990).

2.7.1.3 High-concentrating systems

High-concentrating reactors are usually sun-tracking systems, which tend to have concentrating ratios between 100 and 10,000. Instead of having a linear focus, they have a single focus point, which explains their high concentrating powers. Examples of high-concentrating reactors are parabolic dishes, central tower systems and solar furnaces. High-concentrating systems are generally not employed for photocatalytic degradation of chemical pollutants, as temperature does not play a significant role in photocatalysis (Malato Rodríguez *et al.*, 2004), but are yet to be investigated for disinfection purposes as far as the author is aware.

2.7.1.4 Compound Parabolic Collectors (CPCs)

CPCs are a combination of the one-sun systems and trough collectors and are a popular design for solar photocatalytic reactors, having been found to provide the best optics for low concentrating systems (Robert *et al.*, 2002). Although they can concentrate radiation, they are static and can collect diffuse, as well as direct, radiation. The reactor generally consists of a curved reflecting surface, as shown in Figure 2.20, directing radiation into a tubular absorber. Although the incident radiation on these reactors is very similar to that on non-concentrating reactors, the tube circumference is illuminated, meaning that they perform as well as simple tubular photoreactors (McLoughlin, 2006).

CPCs capture all radiation, be it diffuse or direct, that falls on the aperture area within the acceptance angle, $2\theta_a$. Optimum half acceptance angles are between 60° and 90° .

The main advantages of CPCs, cited by Sichel *et al.* (2005), are as follows:

- Due to the fact that both direct and diffuse radiation are captured (in contrast to most solar tracking systems, they have a high energy efficiency).
- Pressurised systems can be constructed with CPC reactors, allowing for, for example, an increase in dissolved oxygen.
- Turbulent flow can be achieved, which allows for higher levels of dissolved oxygen.
- CPCs are very cost-effective, compared to other medium-concentrating systems.

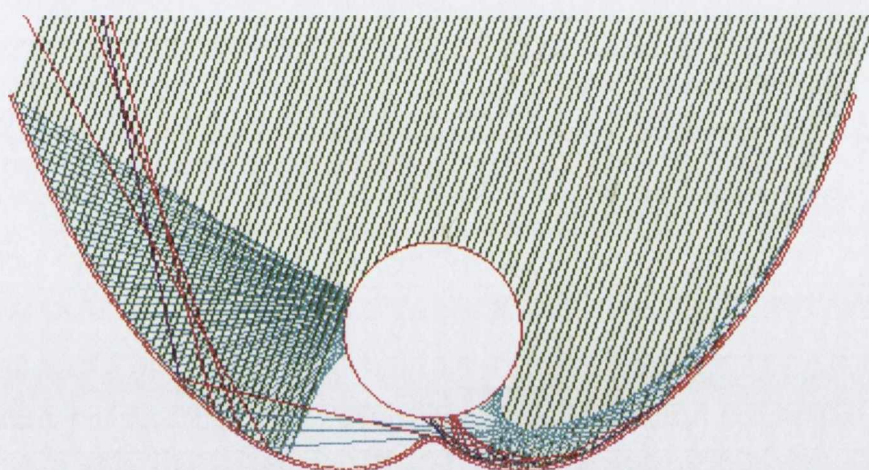


Figure 2.20 Profile of a CPC reactor (www.mech.tohoku.ac.jp/.../E_cpc/non-image-e.html)

2.7.2 The design of CPCs for solar photocatalysis

2.7.2.1 *Tubing material*

The tubing through which the water is passed must obviously be transmissive to UV light and weather resistant, as well as economical. This limits the selection of materials somewhat, with glass and fluoropolymers being the most attractive options. Glass is more commonly used, due to its ability to withstand higher pressures, meaning that the tubes can be much thinner than those made with fluoropolymers, improving transmittance. Only glass with a low iron content should be used, as high iron content in glass leads to absorption of UV light.

2.7.2.2 Reflector material

The ideal properties for a material used as the reflective surface in a photocatalytic reactor are that it is highly reflective between the frequencies of 300 and 400 nm, it is weather resistant and it is relatively inexpensive (Malato Rodríguez *et al.*, 2004). Silver, which is commonly used as a reflective surface in many applications, fails the first and third of these criteria. Instead, aluminium was found to be the most suitable as it is the only metal that is highly reflective in the ultraviolet range (Malato Rodríguez *et al.*, 2004). Aluminium surfaces are prone to oxidation and must therefore be covered in a protective material. Glass is known to filter UV light, so special aluminised plastics and acrylic enamels have been developed.

2.7.2.3 Tube geometry

As mentioned in Section 2.7.2.1, reactor tubes must be kept as thin as possible to optimise transmittance. The diameter of tubes is also crucial for a similar reason, as photocatalyst suspensions in water will absorb and scatter incident light and cells closer to the centre of tubing will receive less light than those near the tube wall. The length and diameter of tubing must also be designed in such a way as to ensure uniform, turbulent flow and to avoid sedimentation of the photocatalyst. Essentially, the designer must ensure that each particle or organism flowing through the reactor receives an adequate UV dose that is similar to that of every other particle/organism. An absolute minimum diameter of 20 – 25 mm was set down for tube diameter in reactors employing suspended photocatalyst, while 25 – 50 mm was seen as ideal (Malato Rodríguez *et al.*, 2004):

2.7.2.4 Flow rate

A turbulent flow regime should be ensured by using a flow rate with corresponding Reynold's number higher than 4000. The turbulent flow ensures that each particle/organism will receive a similar UV dose on passing through the system and will ensure a more uniform concentration of both catalyst and target. It also allows for higher levels of dissolved oxygen in the water, which is important for photocatalytic disinfection, as outlined in Section 2.5.6.3.

2.7.2.5 Optical configuration

Computer methods can be used to predict and optimise the optical characteristics of a reactor design, such as the semi-angle of acceptance, cut-off angle and optical gap. Factors to be considered when modelling are optical loss due to tube thickness and refraction of the light when passing through the water. More on the modelling of reactors is presented in Section 2.7.5.

2.7.3 Suspended vs Fixed Photocatalysts in Reactors

Most work so far has been carried out using TiO_2 in suspension in reactors, as these slurry systems are generally very efficient. The advantages of these systems in comparison to fixed systems include: a lower pressure drop through the reactor; a larger photocatalyst surface area available for adsorption and reaction; and good mass transfer of contaminants from fluid to photocatalyst (Alfano *et al.*, 2000). However, due to the fact that most titanium dioxide powders used in photocatalysis contain sub-micron particles, the post-treatment removal of the photocatalyst is very difficult and highly expensive. Although some authors have investigated alternative methods of photocatalyst removal (Blanco *et al.*, 2001), immobilised photocatalyst reactor systems are becoming ever more popular. Fixed photocatalysts have been proven to be less efficient on a number of occasions against certain microorganisms (Bideau *et al.*, 1995, Rincón *et al.*, 2003 & 2004, Fernández-Ibáñez *et al.*, 2005), but cannot be assumed to be less efficient against all microorganisms, as the reaction mechanisms are not as simple as for the removal of organic pollutants (Blanco-Galvez *et al.*, 2007).

2.7.4 Previous work on reactors

Early studies on the efficiency of introducing titanium dioxide into photoreactors for disinfection were carried out by Zhang *et al.* (1994) and Block *et al.* (1997)

An early immobilised TiO_2 reactor was developed by Ireland *et al.* (1993), this consisted of a UV lamp wrapped with TiO_2 -coated fibreglass. This reactor showed good photocatalytic performance, but the authors highlighted the fact that inorganic radical scavengers can have a detrimental effect on the process and that some catalyst-fouling was suspected.

Vidal *et al.* (1999) were among the first to conduct a pilot plant study about water disinfection using TiO₂ photocatalysis. They employed a CPC with TiO₂ in suspension and operated it in combined flow-through and batch mode. The results showed a 5-log decrease in bacterial (*E. coli* and *Enterococcus faecalis*) concentration after 30 min of treatment. The authors then carried out a cost analysis for the system and, although at the time of print the costs were higher than for conventional technologies, potentially greater cost reductions had been investigated. It must be noted that this reactor was designed for detoxification as well as disinfection, thus driving up the cost.

McLoughlin *et al.* (2004) compared three different types of reactors for solar disinfection, before introducing TiO₂-coated rods to investigate the photocatalytic effect. The compound parabolic collector was found to be more efficient than the parabolic and V-groove collectors, while the introduction of the photocatalyst brought about enhanced results.

Very few photocatalytic disinfection reactors are in use currently for practical applications. One such is the SOLWATER system in Argentina. This reactor combines photocatalysis and photosensitisation to treat polluted water in a village in the Tucumán area of the country. A schematic diagram of the reactor system is shown in Figure 2.21.

2.7.4.1 Engineering design for rural water supply.

In order to apply the results of research into reactors to a practical solar disinfection system, a number of parameters must be taken into consideration, namely:

- The target population
- The per capita daily water requirement
- The amount and intensity of solar radiation received per day
- The threshold intensity for disinfection
- The system flow rate
- The concentration of contaminants
- The disinfection rate coefficient

From these, the flow-rate and size of the required reactor system can be calculated. An example of this is outlined in the case study below.

Case Study: Continuous Flow Disinfection System in Kenya

In a paper by Gill & Price (2010), a solar disinfection system was designed for a village in Kenya with a population of 500. It was calculated from surveys that each inhabitant would require a total of 10 L of water per day. From intensity measurements, it was found that there would be a minimum of 8 hours a day during which the solar radiation would exceed 25 W/m², a conservative value for the threshold intensity for disinfection. A rate constant of 1.7 L/kJ_{UV} had been calculated from previous experiments and a conservative pollution value of 10⁵ CFU/mL was assumed. From this and the required flow rate of 10.4 L/min (the total water requirement/the number of minutes of sunshine), it was calculated that 128 m of glass tubing, i.e. 8 reactor units were required.

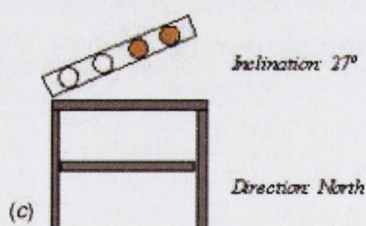
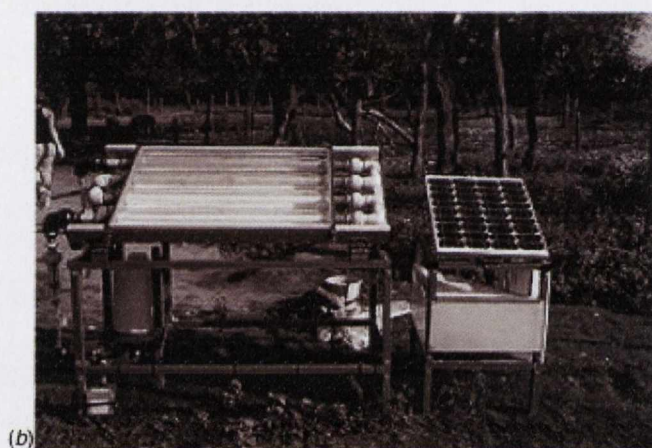
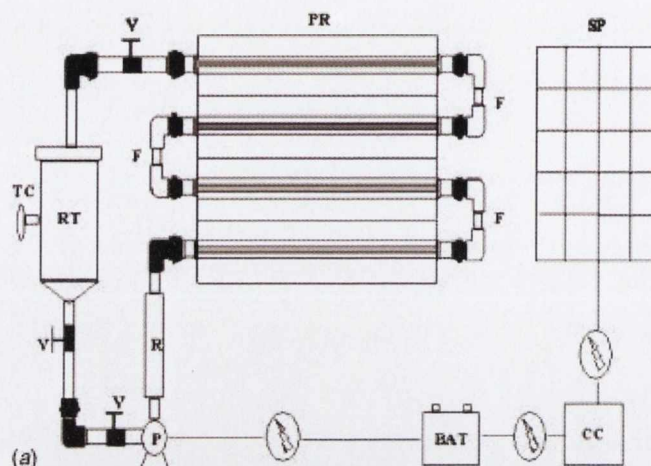


Figure 2.21 SOLWATER reactor configuration (a) flowchart of the reactor; the two lower tubes contain the photocatalyst and the upper two the photosensitizer. PR, Photoreactor; SP, Solar Panel; BAT, Battery; CC, Charge Controller; P, Pump; V, Valve; RT, Recirculation Tank; TC, Thermocouple; R, Rotameter; F, Fittings. (b) View of the reactor on site. (c) Schematic side view (Navntoft *et al.*, 2007).

The reactor consists of two tubes containing TiO_2 -coated paper and two tubes containing a supported photosensitiser. The tubes are in series and mounted on a CPC collector.

It has been found that after 4 h of operation in batch mode on a sunny day, and 5-6 h on a cloudy day, faecal coliforms and *Enterococcus faecalis* are completely destroyed, with no trace of them being found even 24 h after the conclusion of the experiment. The survival of some total coliforms has been observed, possible reasons being the decrease in ambient temperature, the exhaustion of catalyst and sensitiser and the decrease in solar irradiance.

2.7.5. The modelling of reactor systems

Currently, UV and solar reactor design is almost exclusively based on experimental methods, but computational fluid dynamics (CFD) is being used more and more to optimise systems, greatly reducing the need for physical prototypes and testing. The primary limitation of most such processes appears to be the presence of high-velocity, low-turbulence critical flow paths near channel walls, microorganisms in which receive an insufficient UV dose for disinfection to be achieved. Using CFD, the fraction of organisms receiving low doses can be minimised to achieve optimum efficiency.

The FLUENT software programme is used widely for the solution of transport equations in continuous phase models. However, it also allows the user to model a discrete second phase, consisting of spherical particles (which can be used to represent bacteria or photocatalyst particles), in a Lagrangian frame of reference. The programme is capable of computing the trajectories of these particles and can predict the effects of turbulence on them (FLUENT, 2005).

Although there currently appears to be no literature concerning the use of CFD for photocatalytic reactors, much work has been done in the computational modelling of fluid flow in open-channel UV reactor systems, using both the Eulerian and Lagrangian approaches. With the Lagrangian approach, particle tracking methods are used and the UV dose for each tracked particle is calculated by integrating the radiation intensity data along the path traced by the particle with respect to time (Chiu *et al.*, 1999). The Eulerian procedure involves solving equations of radiation distribution, momentum conservation, mass conservation and species mass conservation in order to work out the velocity field, radiant energy field and concentration profile of microorganisms at steady state conditions. All the species components are considered as part of the continuous phase. The Eulerian method looks to solve the

conservation equation of the microorganisms in each control volume of the reactor, whereas the Lagrangian approach concentrates on the trajectory of the particles in the medium (Elyasi and Taghipour, 2006)

In the computer-aided modelling of reactors, the Navier-Stokes equations and extensions thereof are typically solved using numerical estimation procedures to account for turbulence.

The dose received by each particle can be calculated by integrating fluence rate values with respect to time:

$$Dose(P) = \int_0^T I(t) dt \cong \sum I \Delta t \quad (\text{Eqn. 2.19})$$

Dose(P) = UV dose for particle P [J/m²]

I(t) = fluence rate [W/m²]

(Ducoste *et al.*, 2007)

Once the dose received by each particle has been calculated, dose distribution calculations can be undertaken by organising dose values into discrete bin sizes. Thus, a dose-distribution expression, P(D_i) can be formulated, representing the probability that a microorganism will receive a certain UV dose in traversing the reactor system. From this, as well as a knowledge of batch disinfection kinetics, an expression for microbial inactivation can be established:

$$\Phi_{out} = \sum_i \Phi_{BR}(D_i) P(D_i) \quad (\text{Eqn. 2.20})$$

$$\Phi_{out} = N/N_0$$

$\Phi_{BR}(D_i)$ = a dose-response relationship describing inactivation kinetics in a reactor

(Chiu *et al.*, 1999)

The dose-response relationship, $\Phi_{BR}(D_i)$, is explained in detail by Chiu *et al.* (1999), who describe it by series-event kinetics. The term ‘event’ is given to a discrete amount of photoinduced damage and there is said to exist an event level, ‘n’, of damage which, when attained, renders an organism inactivated. The concentration of organisms still viable, therefore is said to be the concentration of all those that have reached level n – 1 or less and is represented by:

$$N = \sum_0^{n-1} N_i = N_0 e^{-kI\tau} \sum_0^{n-1} \frac{(kI\tau)^i}{i!} \quad (\text{Eqn. 2.21})$$

N_i = initial microorganism concentration (assumes all organisms are present at event level zero).

This series-event model reduces to the more conventional Chick-Watson kinetics when n is equal to 1.

An alternate method, outlined by Chiu *et al.* (1999), is the segregated flow model, which combines tracer modelling and CFD analysis to deal with non-ideal hydrodynamic behaviour. Tracer studies are used to obtain residence time or exit-age distribution information and individual fluid ‘parcels’ are treated as CMBRs. Each fluid parcel is assumed to remain segregated, i.e. not mixing with other parcels in the reactor, and thus the degree of disinfection in each parcel depends on the residence time (or exit age) of the parcel. Intrinsic kinetic information can be combined with the exit time to predict the effluent concentration. This approach is only valid for first-order kinetics, however, and so is not ideal for disinfection in a system where the intensity varies spatially.

Certain assumptions are usually made in such modelling procedures, such as perfect reflection from boundaries and perfectly spherical particles. The transmission (or absorbance) of the liquid in the reactor is an important parameter, as the gradient of light intensity across the tubes is affected greatly by it. For high transmission liquids, the overall time spent by a particle in a reactor determines its cumulative UV dose, whereas in low transmission liquids, the time spent by each particle in different radial positions must be calculated (Downey *et al.*, 1998).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Photocatalyst

Titanium dioxide was used as the photocatalyst in all of the experiments. The TiO_2 was supplied by the Degussa company and was of the type P25 Aeroxide (80:20 anatase:rutile). For experiments using sterile water, the appropriate mass of TiO_2 was measured out using a four-figure microbalance (Cahn C-33) and mixed with 1 L of distilled water in an autoclave bottle (Figure 3.1). The contents of the bottle were then sterilised by autoclaving [HiClave HV-25 autoclave (HMC, Japan)] for 40 minutes at 121 °C. For other experiments, the appropriate mass of photocatalyst was added to the reservoir and allowed to mix before the experiment was started. In the bench-scale CPC, the mixing time was 140 s, calculated from previous flow regime tracer studies on the same system using Rhodamine (McLoughlin, 2005), while in the full-scale CPC, the mixing time was calculated to be 5 minutes.



Figure 3.1 Titanium Dioxide suspension

3.1.2 Bacteria

Escherichia coli K-12 (ATCC 10798) was used as the bacteria for all experiments and was stored in freeze dried form at -80 °C before being prepared to give an *E. coli* concentration of approximately 1×10^9 CFU/mL, as outlined in section 3.2.1.

3.1.2.1 Growth Media

Luria Agar and Luria Broth (Sigma Aldrich, USA) were used as the growth media for the *Escherichia coli*. The agar was prepared by adding 40 g of powdered agar to 1 L of sterile water in an autoclave bottle. The bottle was autoclaved for 40 min at 121 °C. When the bottle had cooled to about 50 °C, the agar was poured into Petri dishes and the plates were allowed to dry.

3.1.3 Viruses

The three viruses used in the experiments - MS2 (F-specific coliphage), Φ X174 (somatic coliphage) and PR 772 (*Tectiviridae* coliphage) - were supplied by ATCC in freeze-dried form. After hydration in suitable broths, the viruses were stored at 4 °C.

3.1.3.1 Host Bacteria

Three strains of *Escherichia coli* were used as the hosts for the phages. The bacteria were *E. coli* 15597 (MS2), *E. coli* 13706 (Φ X174) and *E. coli* BAA-769 (PR772) and were all supplied by ATCC.

3.1.3.2 Growth Media

The media used were *Escherichia* broth, agar and top agar (MS2); nutrient broth, agar and top agar (Φ X174); and tryptic soy broth, agar and top agar (PR 772). These were all prepared according to ATCC guidelines.

3.1.4 Reactors

Two compound parabolic collectors (CPCs) were used in the experiments, one laboratory scale and one full-scale. Their characteristics are summarised in Table 3.1.

3.1.4.1 Laboratory scale CPC

The laboratory scale compound parabolic reactor (Figure 3.2) consisted of six parallel Pyrex tubes (9.6 mm internal diameter), each 250 mm long, connected by plastic tubing (which was covered during experiments to ensure that only the Pyrex tubes were illuminated). The half acceptance angle of the reflector was 90^0 and the concentration ratio was approximately 1. The closed loop reactor was connected in series to a submersible pump (2.8 L/min, giving a fluid velocity of 0.64 m/s) pumping from a 1 L reservoir which was located in the dark. The Reynolds number for the flow rate was calculated to be 5450 meaning that the flow regime was in the smooth turbulent zone. The water undergoing treatment was continuously recirculated throughout the experiment.

To prove no photocatalyst settling would occur in the reservoir, the velocity gradient (G) was calculated to be 147 s^{-1} , indicating that the photocatalyst was well mixed. The settling velocity of the TiO_2 particles was worked out to be $1.37 \times 10^{-9}\text{ m/s}$ using Stoke's Law, compared to the fluid velocity of 0.64 m/s, meaning that no settlement could occur in the reactor system.

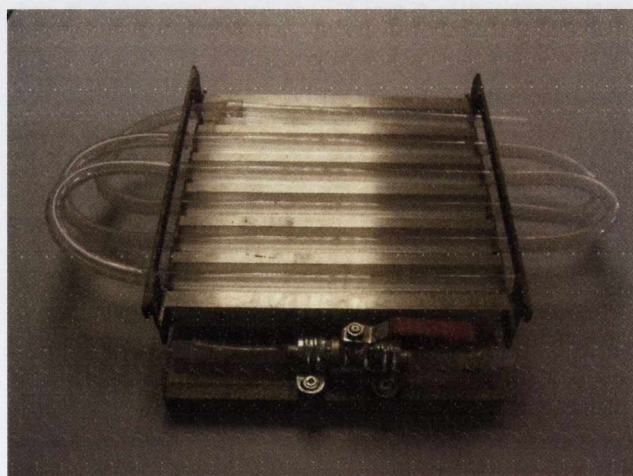


Figure 3.2 Laboratory scale CPC

3.1.4.2 Full-scale CPC

The full-scale CPC (Figure 3.3), obtained from AO SOL (Portugal), consisted of ten parallel glass tubes (23 mm internal diameter), each 1,504 mm long, connected by opaque plastic joints, which were 549 mm (left-hand side of CPC) and 389 mm long (right-hand side). The half acceptance angle of the aluminium reflector was 90^0 and the concentration ratio was 1. The reactor was gravity fed and was connected to two reservoirs, one above the top tube and one on the ground. A peristaltic pump (Watson Marlow, USA) was used to transfer water from the lower reservoir to the upper (Figure 3.4). The characteristics of the two CPCs used are summarized in Table 3.1.



Figure 3.3 Full-scale CPC during mixing of TiO_2

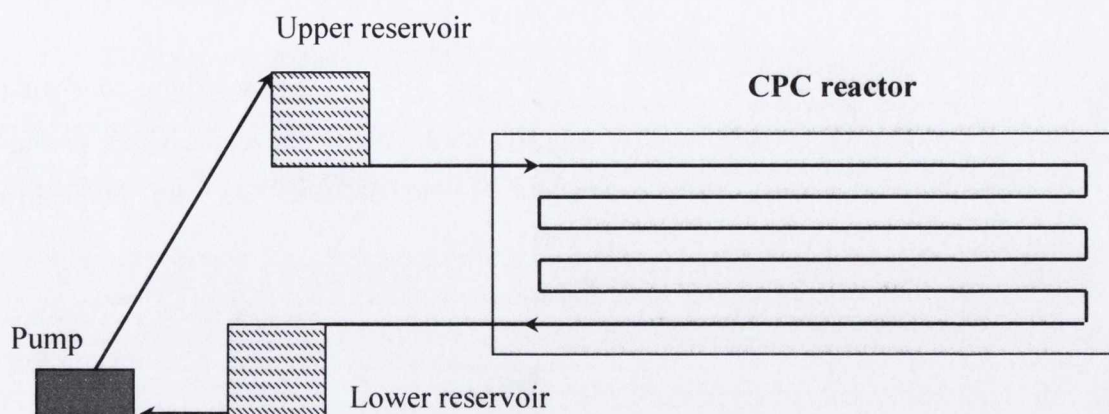


Figure 3.4 Schematic of full-scale reactor set-up

	Bench-scale	Full-scale
Profile	Compound parabolic	Compound parabolic
Half acceptance angle	90 °	90 °
Concentration ratio	~ 1	~ 1
Number of tubes	6	10
Tube length (mm)	250	1504
Internal diameter of tube (mm)	9.6	46
Total irradiated area (m ²)	0.057	2.31
Total circulating volume (L)	1	32
Total irradiated volume (L)	0.108	22.5
Reynolds number of flow	5450	4480

Table 3.1 Reactor characteristics

3.1.5 UV Lamp

The lamp used in the experiments was a 75 W Philips HB175 facial solarium, consisting of four parallel UVA tubes (Figure 3.5). It was mounted 30 cm above the CPC, giving an intensity of 15 W/m^2 at all parts of the collector. Emission spectrum data were obtained from the manufacturer and are displayed in Figure 3.6.



Figure 3.5 UV lamp

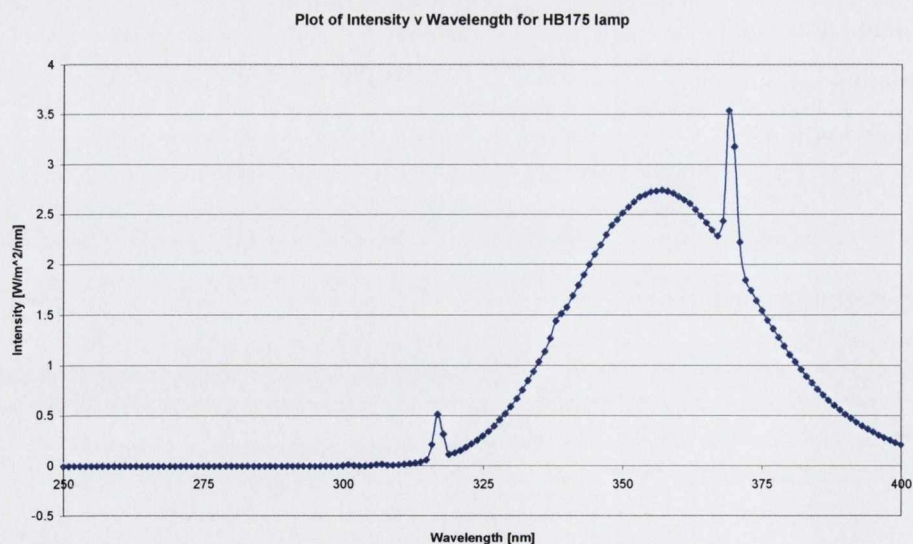


Figure 3.6 Emission spectrum of lamp, plotted with information obtained from Philips.

3.1.6 Radiometer

Light intensity levels were measured using a PMA2100 radiometer (Solarlight, USA), with UVA and UVB sensors. The radiometer was set to record the UVA and UVB intensity in W/m^2 at intervals of one minute. The radiometer was calibrated once yearly by the manufacturers.

3.1.7 Transmission Electron Microscope.

Cells were examined using a Jeol 2100 Transmission Electron Microscope (TEM), housed in the Centre for Microscopy and Analysis (CMA), Trinity College Dublin. The TEM uses a 200 kV accelerating voltage and has a Lanthanum Hexaboride emission source. 2 kB x 2 kB digital images were taken with an AMT capture system.

3.2 Methods

3.2.1 Bacterial propagation and enumeration

In order to propagate the bacteria, a single colony of *E. coli* was transferred from a streaked plate to 50 ml of Luria broth (Sigma-Aldrich, St. Louis, MO). This was incubated for 18 hours at 37 °C and then centrifuged at 3,000 rpm for 12 minutes. The broth was poured off and replaced with 50 mL of sterile water and the sample was centrifuged again. This water was poured off and again replaced with 50 mL of sterile water and centrifuged. When this water was poured off, a pellet of *E. coli* was left at the bottom of the vial. 5 mL of sterile water was added to this to give an *E. coli* concentration of approximately 1×10^9 CFU/mL.

Serial dilutions of the experimental samples were made by adding 100 μL of sample to 900 μL of sterile water. The number of viable cells in the water samples was determined by plating 20 μL (or 200 μL if a low count was expected) of an appropriate dilution on Luria Agar (Sigma-Aldrich, St. Louis, MO) and counting colonies after incubation at 37°C for 18 hours, using the spread plate method. Quality control and assurance guidelines described in the Standard Methods (APHA, 1999) were strictly followed, while all samples were plated in duplicate. The

method detectable limit (MDL) was 50 CFU/mL, as the minimum cell count for statistical methods was 10.

3.2.2 Viral propagation and titration

The host *E. coli* was rehydrated in the appropriate broth and streaked on an agar plate, which was incubated for 18 hours at 37 °C. A colony of the host *E. coli* for each virus was scraped from a plate into a conical flask containing 100 mL of appropriate broth and grown up in an orbital shaker at 37 °C for 18 hours. 200 µL of the appropriate *E. coli* was added to 3.5 ml of melted top agar, which were kept in sterile tubes at 50 °C in a water bath. The tubes containing the top agar and bacteria were then vortexed for 5 s and poured onto agar plates, pre-warmed at 37 °C in an incubator. The plates were allowed to cool at room temperature. The plates were quartered and two aliquots of 20 µL of each sample dilution were plated. The plates were stored overnight in an incubator at 37 °C and the plaques were counted.

When necessary, phages were amplified by preparing plates as outlined above and vortexing the top agar, plaques and *E. coli*, scraped off the plate after incubation. The supernatant was conserved and a titration was carried out as above to determine the concentration of phage.

3.2.3 Reactor set up

3.2.3.1 Bench-scale CPC

Experiments under Artificial UV Light

The reactor was placed in a non-fluorescent box, below a parallel UV-A light source consisting of four parallel UV tubes (Philips HB 175) and subjected to a light intensity of 15 W/m² [measured using a PMA2100 radiometer (Solarlight, USA)]. The reactor was first disinfected by pumping 0.1 M ethanol around it for five minutes. This was then followed by rinsing with four flushes of sterile water to ensure that no ethanol would remain in the system and lead to unwanted bacteria kill-off during the experimental periods. This rinsing procedure was proven to remove all traces of ethanol in a previous study on the same system (McLoughlin, 2005).

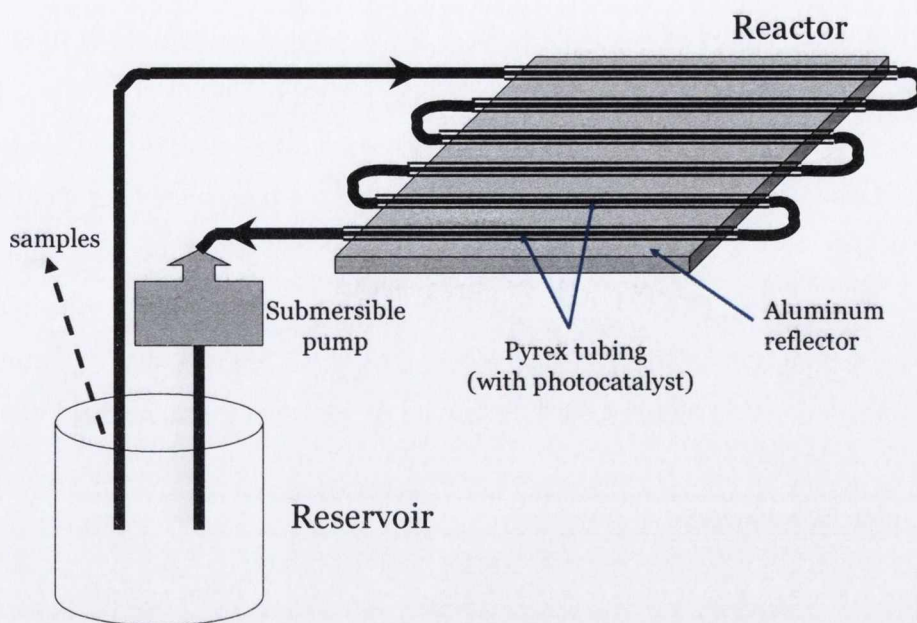


Figure 3.7 Bench-scale CPC set-up under artificial UV light

For bacterial experiments, *E. coli* was prepared as discussed in Section 3.2.1. 1 mL of the bacteria was added to 1 L of sterile water in the reservoir, containing the appropriate concentration of TiO_2 , to give an initial *E. coli* concentration of approximately 1×10^6 CFU/mL. After priming the pump with the sample, the tubes at either end of the reactor system were placed in the reservoir, along with a thermometer (Figure 3.7). The reservoir was placed in a non-fluorescent chamber and the pump was turned on. From previous flow regime tracer studies on the same system using Rhodamine, it was determined that complete mixing of the *E. coli* occurred throughout the system after 140 seconds (McLoughlin, 2005). At this time, a 1 mL sample was taken from the reservoir, using a sterile transfer pipette, as the UV light source was switched on. Samples were then taken at the sample points described below. The sample points were: 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes, with samples also taken at 420 and 1500 minutes in some experiments.

The methodology for the viral experiments under UV light was as above, but with 1 mL of each phage added to the reservoir instead of *E. coli*.

Experiments under Natural Sunlight

The experiments using the bench-scale reactor under natural sunlight were conducted on the roof of the Civil Engineering Building in Trinity College, Dublin (Latitude 53°21'). Two reactors were set up parallel with each other, allowing a photocatalytic and photolytic test to be run simultaneously. The collectors were inclined due south at an angle of incidence equal to the local latitude. UV-A and UV-B intensity were measured using the Solarlight radiometer, with the detectors also directed due south at an angle of 53°21' to the horizontal. As previously, the reservoir for each reactor was placed in a darkened box. A thermometer was placed in each reservoir to monitor the temperature of the water throughout the experiments.

Post-irradiation experiments

The post-irradiation activity of photolysis and photocatalysis was investigated by keeping each sample taken at 37 °C in the dark. As soon as an experimental sample was taken and plated, the 30 mL vial containing the sample was placed in the incubator. These samples were then replated, as detailed in Section 3.2.1, 24 h and 48 h after they had been taken.

3.2.3.2 Full-scale CPC

Experiments were also performed in the full-scale CPC under natural sunlight. The CPC, as described in section 3.1.4.2, was tilted due south at 53°21' to the horizontal, which corresponds to the angle of latitude of the location at which the experiments were undertaken (Trinity College, Dublin, Ireland). The water was allowed to flow by gravity from a covered elevated reservoir through the CPC to a second covered reservoir situated at ground level (Figure 3.4). The water was pumped back to the higher reservoir using a peristaltic pump (Watson Marlow 624S, USA) and the pump flow rate was adjusted to ensure a constant head was achieved. The UV-A and UV-B detectors were attached to the frame of the reactor, which meant they were also inclined at an angle corresponding to the angle of latitude. After ethanol was passed through the system for disinfection purposes, the reactor was rinsed using four flushes of distilled water, followed by the type of water being used in the experiment. The reactor was covered while *E. coli* or phage samples were spiked into the lower reservoir. TiO₂ was also added at this point in the photocatalytic experiments. After five minutes, when complete mixing of the microorganism had occurred, the first sample was taken. 1 mL samples were then taken using a sterile transfer pipette at the following times: 15, 30, 45, 60, 90, 120, 180,

240, 300 and 360 minutes. A thermometer was placed in the lower reservoir and the temperature of the water was noted at each sample time.

3.2.4 Analysis of Results

In order to calculate the concentration of *E. coli* at time t (N_t) in CFU/mL (colony forming units/ml), the following equation was used:

$$N_t = (\text{number of colonies on plate}) \times (\text{dilution factor}) \times 50 \quad (\text{Eqn. 3.1})$$

If 200 μL of sample had been plated without dilution, the concentration was obtained by:

$$N_t = (\text{number of colonies on plate}) \times 5 \quad (\text{Eqn. 3.2})$$

For the phages, the corresponding calculations were:

$$N_t = (\text{number of plaques on plate}) \times (\text{dilution factor}) \times 50 \quad (\text{Eqn. 3.3})$$

$$N_t = (\text{number of plaques on plate}) \times 5 \quad (\text{Eqn. 3.4})$$

Where N_t had units of PFU/ml (plaque forming units/mL).

For a sample taken at time t , the phage concentration (N_t) was divided by the initial concentration (N_0) and the log of this value was plotted against time and/or cumulative dose.

The cumulative UV dose was calculated using the following formula:

$$Q_{uv,n} = Q_{uv,n-1} + \frac{\Delta t_n UV_{G,n} A}{V_T} \quad (\text{Eqn. 3.5})$$

where Q_{UVn} and Q_{UVn-1} are the cumulative irradiated UV energy received per L of sample at times n and $n-1$; Δt_n is the time interval between sampling times; UV_{GN} is the average incident radiation on the irradiated area; A is the irradiated area; and V_T is the total circulating volume.

In the case of the UV lamp, the intensity was a constant 15 W/m² throughout the experiment, so the equation became:

$$Q_{uv,n} = \frac{t_n UV_{G,n} A}{V_T} \quad (\text{Eqn. 3.6})$$

where t_n is the time at which the sample is taken and UV_{GN} is a constant value of 15 W/m².

Error bars representing standard deviation were plotted.

3.2.5 Microscopy preparation

In Chapter 4, a microscopic analysis of *E. coli* samples was undertaken to determine the mechanism of cell inactivation in the presence and absence of TiO₂. The cell preparation and subsequent microscopy was performed in the Centre for Microscopy and Analysis, Trinity College Dublin.

0.6 ml samples of the bacteria to be analysed were placed in Eppendorff tubes and 0.6 ml of fixative solution (3 % glutaraldehyde in 0.05 M phosphate buffer) was added. The samples were centrifuged for 5 minutes at 1500 rpm and washed with 0.05 M phosphate buffer to remove any unreacted glutaraldehyde. This was repeated 6 times. The supernatant was discarded and the pellets were cut into 1 mm cubes. A secondary fixative solution (2 % osmium tetroxide in 0.05 M potassium phosphate buffer) was added to the samples and these were agitated for 30 minutes. An alcohol dehydration series was undertaken as follows:

1. 10 % alcohol in water for 10 minutes
2. 30 % alcohol in water for 10 minutes
3. 50 % alcohol in water for 10 minutes
4. 70 % alcohol in water for 10 minutes
5. 95 % alcohol in water for 10 minutes
6. 100 % alcohol for 15 minutes
7. 100 % alcohol for 15 minutes
8. 100 % alcohol for 30 minutes

The samples were then transferred to a transitional fluid (100 % propylene oxide), which is miscible with both alcohol and resin, for 15 minutes and then again for thirty minutes. Resin was made up using 24 g Agar 100 Epoxy resin, 16 g DDSA Hardener, 10 g MNA Hardener and 1.5 g BDMA Accelerator. A 50 % resin solution (10 ml resin plus 10 ml propylene oxide) was added to the samples. This was agitated on the rotator for three hours. This was then replaced with a 100 % resin solution and again agitated for three hours. Samples were removed from the glass vials using an applicator stick and the excess resin was removed on filter paper. The samples were transferred to pans containing fresh resin. The pans were placed in a vacuum oven and the vacuum was turned on for ten minutes. The oven was restored to ambient pressure and the samples were left in at 60 °C for 24 hours. Samples were cut in a microtome and stained using 0.5 % aqueous uranyl acetate and one drop of Reynold's lead citrate. The grids were washed and dried and observed under the microscope.

4. POST-IRRADIATION EVENTS IN THE ABSENCE AND PRESENCE OF TiO₂

4.1 Introduction

The use of sunlight alone as a means to disinfect water has been shown to be effective against various microorganisms in several studies (McGuigan *et al.*, 1999, Smith *et al.*, 2000), although a question still hangs over its practical viability due to post irradiation bacterial regrowth, a phenomenon that has been observed by some authors (Rincón & Pulgarin, 2004a) and has been discussed in Section 2.5.5.

The mechanism of cell death in the presence of TiO₂ as a photocatalyst has been described in Section 2.5.2. In summary, the mechanism is understood to be as follows: the hydroxyl radical generated at the TiO₂ surface makes contact with intact cells. After eliminating the cell wall protection, oxidative damage takes place on the underlying cytoplasmic membrane. Photocatalytic activity progressively increases the cell permeability and subsequently allows the free efflux of intracellular contents that eventually leads to cell death. The cell membrane is the primary site of reactive oxygen species attack and oxidative attack of the cell membrane leads to lipid peroxidation (Dunlop *et al.*, 2002). The cell membrane provides an attachment site for cellular respiration and when it is damaged beyond repair, respiration ceases. Cells with a damaged cell wall can still be viable but it is the combination of cell membrane damage and further internal cellular oxidative attack that leads to cell death.

As reported in Section 2.5.5, one of the potential benefits of using a photocatalyst reported by some authors is that the photocatalytic disinfection mechanism prevents any re-growth of the microorganism post-irradiation if the water is subsequently stored in the dark (Rincón & Pulgarin, 2004a). The same authors have conducted studies into post-irradiation effects and have introduced the concept of effective disinfection time (EDT), the time required to inactivate bacteria such that no regrowth occurs in a subsequent 24 (or 48) hour dark period. They also showed that post-irradiation behaviour in bacteria is similar for fixed and suspended TiO₂ systems (Rincón & Pulgarin, 2004b). Other experiments conducted to investigate the phenomenon of regrowth in water treated by both SODIS (solar disinfection) and SPC-DIS found that neither faecal nor total coliforms showed any sign of regrowth when kept in the dark after photocatalytic treatment, whereas bacterial regrowth was observed after SODIS

(Gelover *et al.*, 2006). It has been seen, however, that if cells are exposed to solar UVA of high enough intensity for a long enough period of time, then they are unable to repair the membrane damage inflicted on them (Berney *et al.*, 2006a) even though they may initially show up as still viable. It was also found that solar disinfection has a sub-lethal, as well as a lethal effect, whereby membrane damage leads to bacterial growth delay after irradiation (Berney *et al.*, 2007, Kubitschek & Doyle, 1981, Pizarro & Orce, 1988).

Studies on the photocatalytic prevention of regrowth have usually been conducted under high intensity light – for example 400 W/m² and 1,000 W/m² lamps (Rincón & Pulgarin, 2004a, 2003), although some solar experiments have been conducted at lower intensities (Rincón & Pulgarin, 2007), albeit at a more southerly latitude than the work presented in this chapter. Intensities above 800 W/m² were required in photocatalytic-SODIS experiments for effective disinfection and prevention of subsequent regrowth (Gelover *et al.*, 2006). This chapter investigates bacterial inactivation and post-irradiation events under a low intensity light and at relatively low sunlight intensity levels.

4.2 Materials and Methods

4.2.1 Experiments under artificial UV light

The bench-scale CPC detailed in Section 3.1.4.1 was used for all experiments under artificial UV light, and the methods were as described in Section 3.2.3.1. Preliminary experiments were conducted under the UV lamp to decide upon a suitable concentration of TiO₂ to be used in the experiments. The reactor was dosed with an initial concentration of 1×10^6 CFU/mL of *E. coli* K-12, and with suspended TiO₂ in the following concentrations: 0 mg/L, 3 mg/L, 6 mg/L, 9 mg/L, 15 mg/L, 50 mg/L, 250 mg/L and 1,000 mg/L. From these, a suitable concentration of 50 mg/L of TiO₂ was chosen for subsequent photocatalytic experiments. Each experiment under UV light was conducted three times, to ensure reproducibility and accuracy of results. Error bars representing standard deviation are included where appropriate.

The data were compared by plotting graphs of log₁₀ bacterial decay vs time and cumulative UV dose (Eqns. 3.5 & 3.6). Post-irradiation data were plotted using bar graphs of bacterial

concentration (in CFU/ml, on a logarithmic scale) vs time. The data for each of the post-irradiation samples (0 h, 2 h, 24 h and 48 h) were plotted at each time step.

4.2.2 Experiments under natural sunlight

The experiments outlined in Section 2.3.1 were repeated under natural sunlight on the roof of the Civil Engineering Building in Trinity College, Dublin (Latitude 53°21') on 18th June (Trial 1), 18th September (Trial 2) and 26th September 2008 (Trial 3). Two reactors were set up, allowing a photocatalytic and photolytic test to be run simultaneously. The parabolic collectors were inclined due south towards the sun at an angle of incidence equal to the local latitude. The tests were again carried out for 0 and 50 mg/L of TiO₂ in suspension. UV-A and UV-B intensity was measured using the Solarlight[®] radiometer, tilted at 53°21' to the south, with measurements taken every minute throughout the duration of the experiment.

The average values of total UV intensity were 33.7 W/m², 15.3 W/m² and 22.0 W/m² for Trials 1, 2 and 3, respectively. The UV-B pattern closely followed the UV-A pattern on all three days but at much lower intensities – by a factor of 2.3×10^{-3} , on average.

4.2.3 Experiments investigating the influence of water type on post-irradiation behaviour

The use of sterile or distilled water can give results which may be misleading when applied to practical applications. It is reported that in distilled water, in the absence of certain ions, bacterial cell walls are weakened by the loss of magnesium and calcium ions. This renders the cell wall less able to cope with the osmotic pressure difference between the water and the interior of the cell and thus the bacterium becomes more susceptible to disinfection (Malato *et al.*, 2009). Also, post-irradiation effects of photocatalysis have shown very different results in distilled water and crude river water. Wist *et al.* (2002) witnessed no bacterial recovery after the photocatalytic treatment of distilled water, whereas the phenomenon was witnessed when they repeated the experiments using river water. To this end, a further regime of experiments was carried out in which three different water types were used. These experiments were performed in the full-scale CPC under natural sunlight, as detailed in Section 3.2.3.2.

The three types of water used were: distilled water, prepared using a conventional laboratory still; rainwater, collected in a domestic rainwater tank in Dublin, Ireland; and river water, collected from the River Liffey at Islandbridge, Co. Dublin. The three water types were tested for potassium, manganese, magnesium, aluminium, iron and total phosphorous using an Inductively Coupled Plasma Atomic Emission Spectroscope (ICP-AES, Varian Liberty Series II). Acidification was performed by pipetting 10 mL of each sample into separate containers, each containing 1 mL of 69 % nitric acid. These were then refrigerated at 4 °C for two hours to allow the samples to set, before analysis was undertaken (Table 4.1).

Water Type	Al [mg/L]	K [mg/L]	Mg [mg/L]	Mn [mg/L]	Fe [mg/L]	Total P [mg/L]	TOC [mg/L]	Alkalinity [mg/L CaCO ₃]
Distilled	0	0	0	0	0.01166	0.03248	1.5	Trace
Rainwater	0.017	4.5147	4.2289	0.069	4.4987	0.39973	3.1	7.12
River	0.067	3.8389	10.373	0.0534	0.24	0.09534	7.4	214

Table 4.1 Chemical analysis of different water types used.

As discussed in section 3.2.4, plots were made of log₁₀ bacterial inactivation vs time and cumulative dose. Post irradiation data were also plotted as before (Section 4.2.1).

The experiments outlined in Sections 4.2.1, 4.2.2 and 4.2.3 were also repeated in the dark as control experiments.

4.2.4 Transmission Electron Microscopy

Parallel experiments were conducted in which 35 mL of *E. coli* were exposed to artificial UV light, in the presence and absence of 50 mg/L of TiO₂. Bacteria were placed in a 1 L Pyrex bottle which was laid on its side and agitated at regular intervals. Because of the nature of the microscopy preparation, whereby a pellet of bacteria was required from each sample, a much higher concentration of *E. coli* (1 x 10⁹ CFU/mL) had to be used than in previous experiments. This meant that a high degree of light attenuation would occur due to the increased turbidity. Therefore, only 35 mL of *E. coli* was used, in order to keep the layer of bacteria as shallow as possible to allow for increased light penetration. The UV lamp was brought closer to the bottle than it had been for the CPC, to achieve the same incident radiation on the bacteria. The

exposure time was chosen to be 15 h, to compensate for the increased turbidity of the sample and to ensure a high degree of disinfection occurred. Samples were taken at regular time intervals, plated to determine the cell count and then prepared for microscope analysis (Section 3.2.5).

4.3. Results and discussion

4.3.1 Inactivation kinetics with respect to photocatalyst concentration.

The results from the preliminary experiments varying the TiO_2 concentrations in the CPC reactor under the UV lamp can be seen in Figure 4.1. The increase in photocatalyst concentration brought about higher reaction rates, with the exception of the 9 mg/L and the 1,000 mg/L samples. It is suspected that a shading effect, where TiO_2 particles shade target organisms as well as each other from the UV light source, was responsible for this drop in efficiency at 9 mg/L. This is similar to results obtained previously (McLoughlin *et al.*, 2004a), and the shading phenomenon has been observed before by many authors (Block *et al.*, 1997, Fernández *et al.*, 2005). It was found, for example, that a concentration of, for example, 9 mg/L of TiO_2 at a path length of 30 mm will reduce the sum of absorbed and scattered light by about 15-20% in certain reactor set-ups (Malato-Rodríguez *et al.*, 2004). As incident light intensity is an important parameter in photocatalysis, this shading has a negative effect on disinfection. Hence, the net disinfection efficiency caused by the combination between the UV effect and photocatalytic effect is somewhat muted at around a concentration of 9 mg/L. However, at higher concentrations, the proximity of TiO_2 particles to target organisms begins to dominate the disinfection process whereby the photocatalytic effect overwhelms any synergistic benefits with the solar effect and reaction rates thus rise again with concentration. This again corroborates with previous observations, which have found that direct UV light attack on bacteria at low TiO_2 concentrations is more important than the photocatalytic effect with the limiting factors being the defense and repair mechanisms of bacteria towards the incident photons. At higher concentrations the photocatalytic production of $\bullet\text{OH}$ radicals begins to dominate the process (Rincón & Pulgarin, 2003).

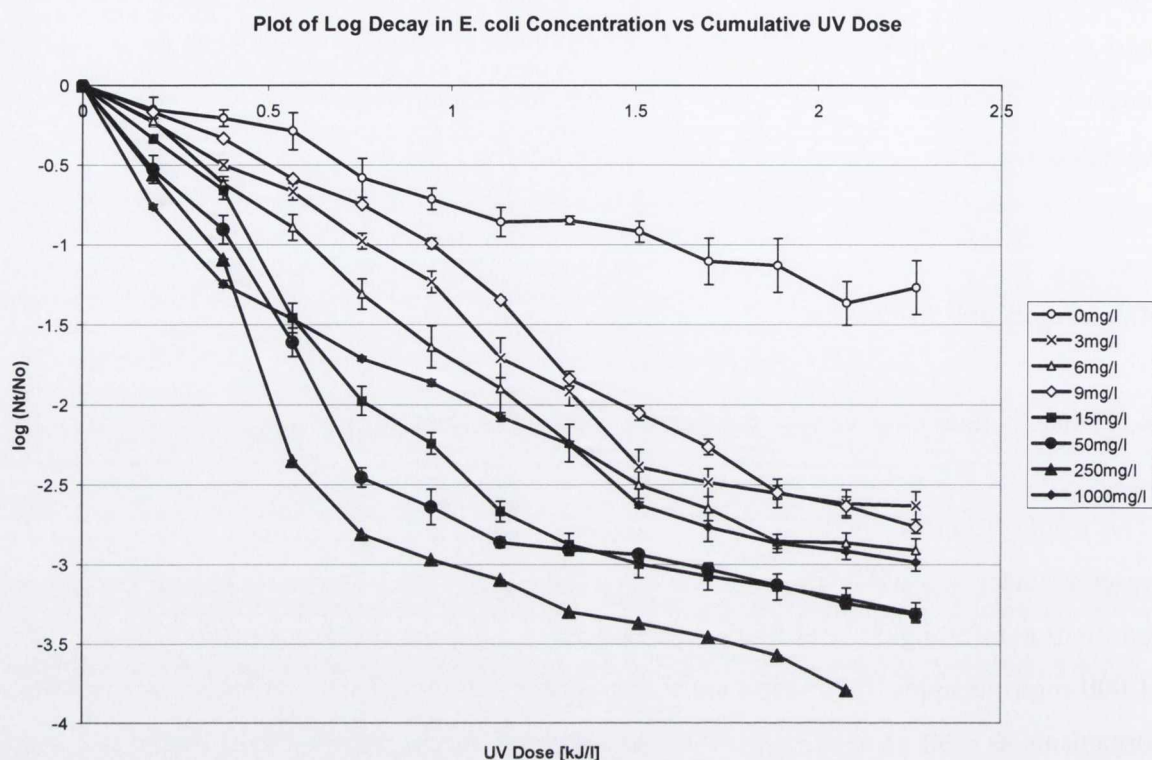


Figure 4.1 Plot of bacterial inactivation against cumulative UV dose for different concentrations of TiO_2

It is interesting to note that at a TiO_2 concentration of 1,000 mg/L the disinfection efficiency dropped away again compared to the nearest high TiO_2 doses of 250 mg/L and 50 mg/L. It is hypothesised that this was due to the photocatalyst particles shading each other at such a high concentration, as well as the target organisms from the incident UV, meaning that the net production of radicals was reduced. At these higher concentrations, the bacteria kill-off in the first five minutes was markedly more pronounced than for the other lower concentrations, but subsequently slowed up significantly, i.e. at higher concentrations, the disinfection kinetics were biphasic (see Table 4.2). At the beginning of the illumination, there was a high concentration of both target organism particles and photocatalyst particles. Therefore, it is likely that many *E. coli* cells were in close proximity to the photocatalyst, causing rapid disinfection. As the number of viable bacteria diminished, so the likelihood of contact between TiO_2 (and thus hydroxyl radicals) and viable *E. coli* decreases, leading to the slower second phase. At lower concentrations, the kinetics were more linear, as the direct photolysis dominates the disinfection. This raises the question of the effect of initial target organism

concentration on disinfection performance (as opposed to catalyst concentration), which should be an area of future research.

From these experiments, 50 mg/L of photocatalyst was chosen as a suitable concentration for subsequent experiments due to it being highly efficient while still a relatively low concentration and because it leads to disinfection over a timeframe suited to our sampling techniques.

TiO ₂ conc [mg/L]	1 st phase k _t [min ⁻¹]	2 nd phase k _t [min ⁻¹]	1 st phase k _{UV} [[kJ/L] ⁻¹]	2 nd phase k _{UV} [[kJ/L] ⁻¹]
0	0.02		0.63	
3	0.05		1.34	
6	0.06		1.49	
9	0.05		1.27	
15	0.07		1.78	
50	0.11	0.02	3.00	0.44
250	0.14	0.03	3.72	0.75
1 000	0.13	0.04	3.44	0.98

Table 4.2 Inactivation rate constants for different concentrations of TiO₂ in terms of time, k_t, and cumulative UV dose, k_{UV}. The inactivation rates are derived from Chick’s Law:

$$\frac{dN}{dt} = -kN \Rightarrow N_t = N_0e^{-k_t t} \tag{Eqn. 4.1}$$

$$\frac{dN}{dQ_{UV}} = -kN \Rightarrow N_t = N_0e^{-k_{UV}Q_{UV}} \tag{Eqn. 4.2}$$

4.3.2 Bacterial Inactivation in the absence and presence of TiO₂

As expected, very little bacteria die-off was observed during the dark experiments [Figure 4.2(a)]. The die-off when TiO₂ was present was slightly higher, which could be due to the agglomeration and sedimentation of bacteria cells and catalyst particles in samples just before they were plated (Malato *et al.*, 2009).

For both the UV lamp and sunlight experiments the initial inactivation rate was far higher when TiO₂ is present, as shown in Figure 4.2 (Note: in Figure 4.2, the straight line to which

each photocatalytic line eventually tends corresponds to the MDL). It can also be seen in Figure 4.2 that the photocatalytic tests show a much more clearly biphasic trend, for reasons explained in Section 4.3.1. Bacterial inactivation by sunlight can be enhanced by a synergistic effect of both UV light and thermal heating above 45 °C (Wegelin *et al.*, 1994), while it has been shown that even at lower temperatures a slight increase in temperature can greatly increase the effectiveness of solar disinfection against certain organisms (Berney *et al.*, 2006b). In these experiments, temperatures did not exceed 30 °C, however, so this effect can be discounted. It must be noted that results found using the small-scale reactor can not be applied directly to larger scale models. A significant limitation of the small-scale CPC that must be mentioned is that the time spent in the dark is high in comparison to the time spent under illumination. It has been shown previously that bacteria can show significant recovery during these dark times and this will lead to a slower inactivation rate than under continuous illumination (Ubomba-Jaswa *et al.*, 2009).

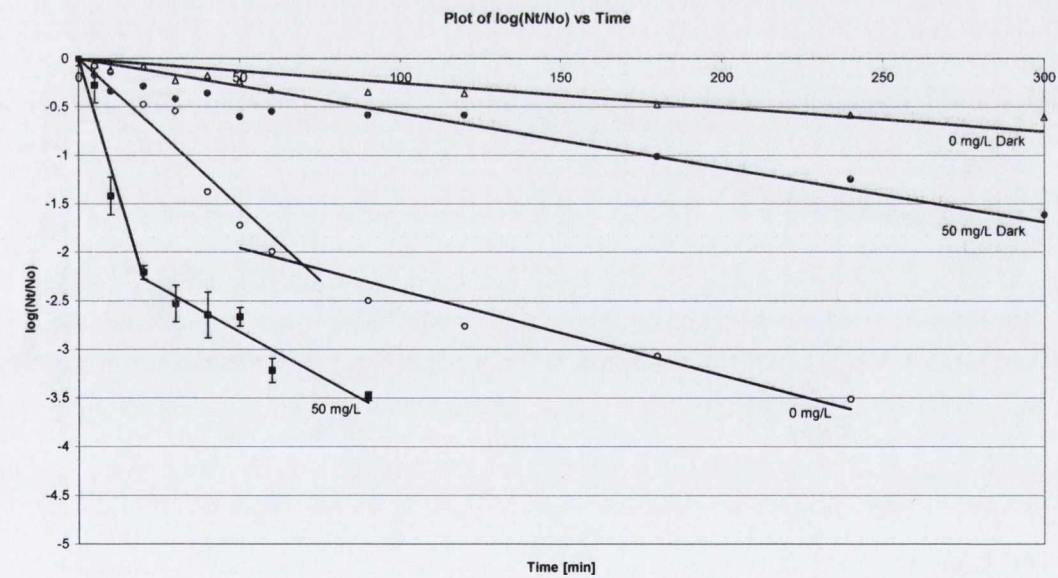
Table 4.3 provides information on the removal of *E. coli* at different stages of the rooftop experiments. Trial 1 had the highest average and peak UV intensities (33.7 W/m² and 50 W/m², respectively), yet showed the slowest inactivation kinetics, both with and without catalyst present. This could be explained, however, by the fact that the initial intensity was lower for Trial 1 than for the other two trials – the average first-hour intensities were 13.9 W/m², 21.8 W/m² and 26.8 W/m², respectively. Indeed, a significant increase in the bacterial inactivation rate was observed after two hours in the photocatalytic part of Trial 1, which coincides with the sharp rise in intensity. The table shows, as previously noted by other research (Rincón & Pulgarin, 2004b), that cumulative dose is not a reliable parameter for predicting disinfection. These authors explained that disinfection and subsequent reactivation are affected by factors such as the relationship between UV and visible intensities, as well as seasonal and daily differences. It is also interesting to note that, although the sunlight intensity is varying, Figures 4.2 (b) – (d) show a smooth relationship between inactivation and time. The authors hypothesise that this may suggest that there is a threshold intensity, above which kinetics are related to time more strongly than to intensity.

All the photocatalytic plots show a tendency for the inactivation kinetics to slow down towards the end of the process, as the active bacteria must compete for oxidative species with inactive bacteria and the products of cell lysis (Rincón & Pulgarin, 2003). In addition, other

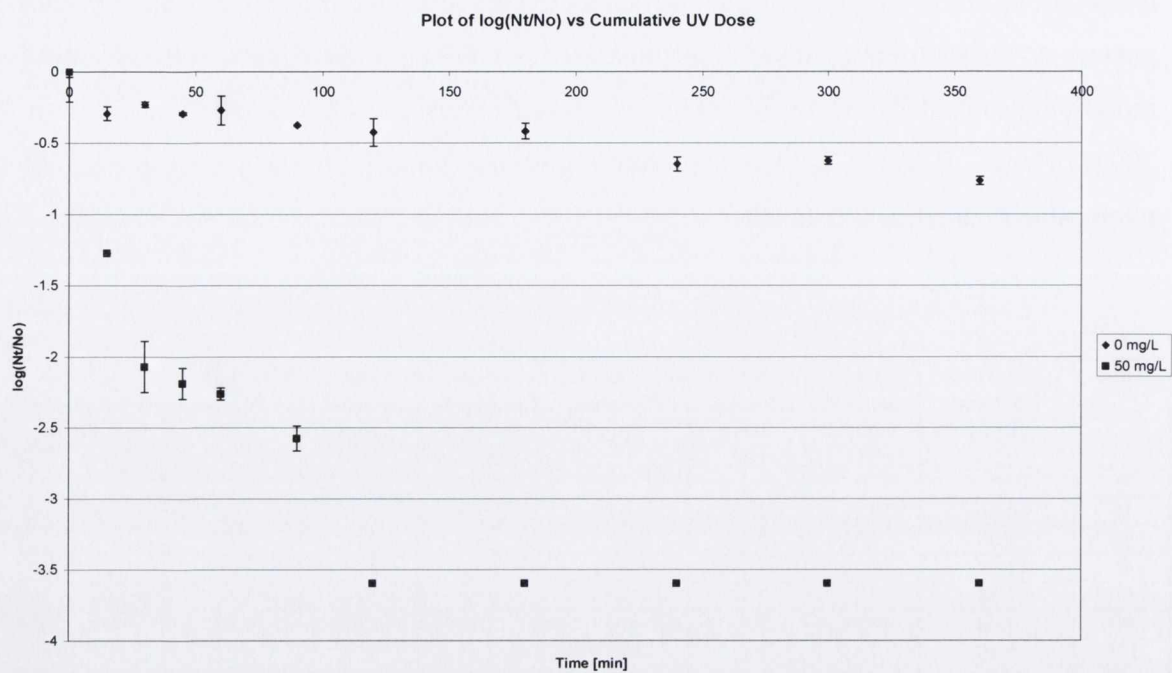
research has discovered a similar tailing effect in their experiments on UV deactivation of *C. parvum* oocysts attributing it to the fact that a subpopulation of the oocysts were genetically or morphologically different to the others and less responsive to UV irradiation (Craik *et al.*, 2001). Hence, it could be the case that some *E. coli* are similarly less susceptible to photocatalytic treatment than others.

Time	Trial 1			Trial 2			Trial 3		
	Cumulative UV Dose [kJ/L]	Log removal without TiO ₂	Log removal with TiO ₂	Cumulative UV Dose [kJ/L]	Log removal without TiO ₂	Log removal with TiO ₂	Cumulative UV Dose [kJ/L]	Log removal without TiO ₂	Log removal with TiO ₂
0	0	0	0	0	0	0	0	0	0
15	0.46	0.29	1.27	0.80	0.13	2.34	1.02	0.21	1.95
30	1.00	0.23	2.07	1.63	0.43	3.66	2.03	0.36	2.32
45	1.57	0.29	2.19	2.47	0.80	4.03	2.99	0.46	2.74
60	2.10	0.27	2.26	3.29	1.35	4.20	4.06	0.95	3.24
90	3.44	0.38	2.58	4.89	1.22	4.51	6.08	0.92	4.35
120	5.89	0.43	3.60	6.29	1.41	4.51	7.88	1.62	4.65
180	13.42	0.42	3.60	9.28	1.70	4.51	11.69	1.94	4.65
240	19.99	0.65	3.60	11.23	1.88	4.51	15.73	2.58	4.65
300	25.58	0.63	3.60	12.91	1.89	4.51	18.26	2.85	4.65
360	26.79	0.77	3.60	13.90	2.03	4.51	20.03	2.99	4.65

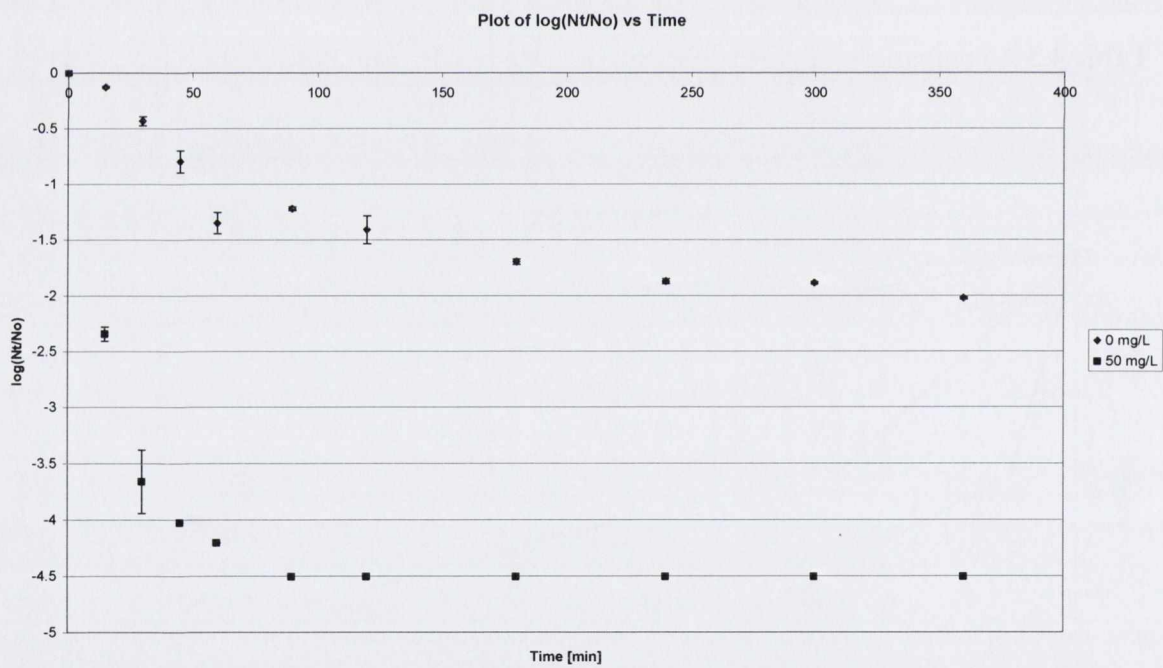
Table 4.3 A comparison of the three trials conducted under natural sunlight



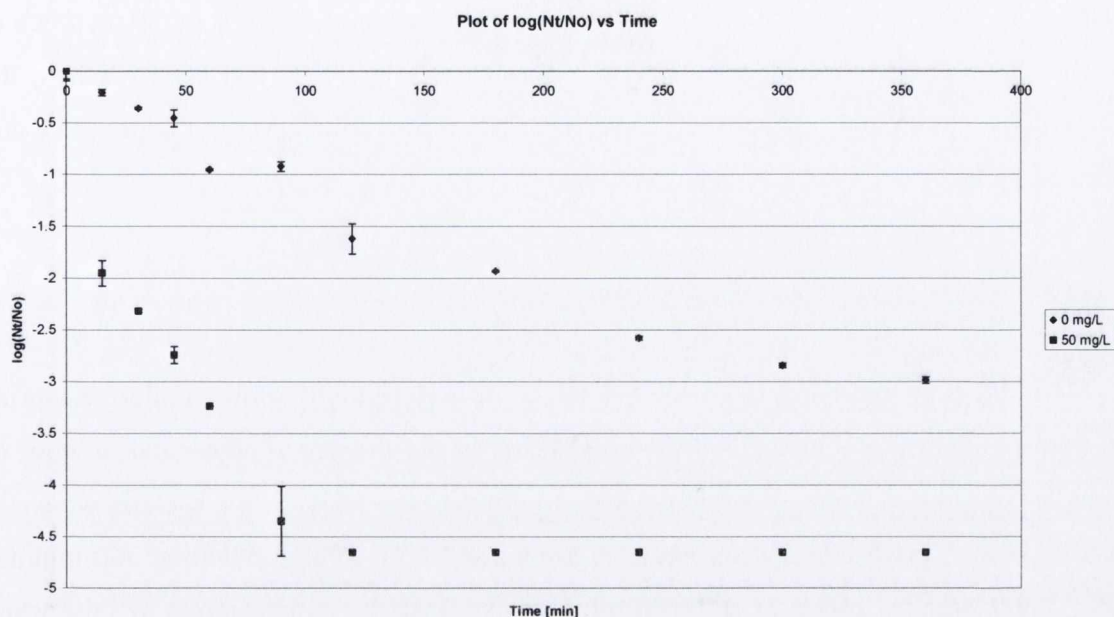
(a)



(b)



(c)



(d)

Figure 4.2 Plots of log bacterial decay against time for (a) test under UV light and in dark (error bars included on just one plot to avoid confusion); (b) Trial 1 under natural sunlight, 18th June 2008, (c) Trial 2 under natural sunlight, 18th September 2008, (d) Trial 3 under natural sunlight, 26th September 2008.

4.3.3 Post-irradiation experiments

4.3.3.1 Post-irradiation events after UV treatment

The experimental results for the trials aimed at determining the survival of bacteria after photolytic and photocatalytic exposure under UV light are shown in Figures 4.3 (a) and (b). These reveal that in neither case did there appear to be any sign of regrowth occurring within a 48 hour post-irradiation period. However, Figure 4.3 (a) shows that in the absence of TiO_2 there were still culturable cells 24 and 48 hours post-irradiation for the sample taken at 60 minutes.

When photocatalyst was introduced, Figure 4.3 (b) shows that after just 10 minutes there were no living cells for the samples tested 2, 24 or 48 hours post-irradiation, indicating a photocatalytic enhancement in terms of the post-irradiation viability of the cells. Hence, the

findings from these tests suggest a far more efficient destruction of the cells in the presence of catalyst. Although the photolytic tests were not run until the MDL had been reached, the findings show a stark contrast between bacterial survival post-irradiation in the presence and absence of TiO_2 .

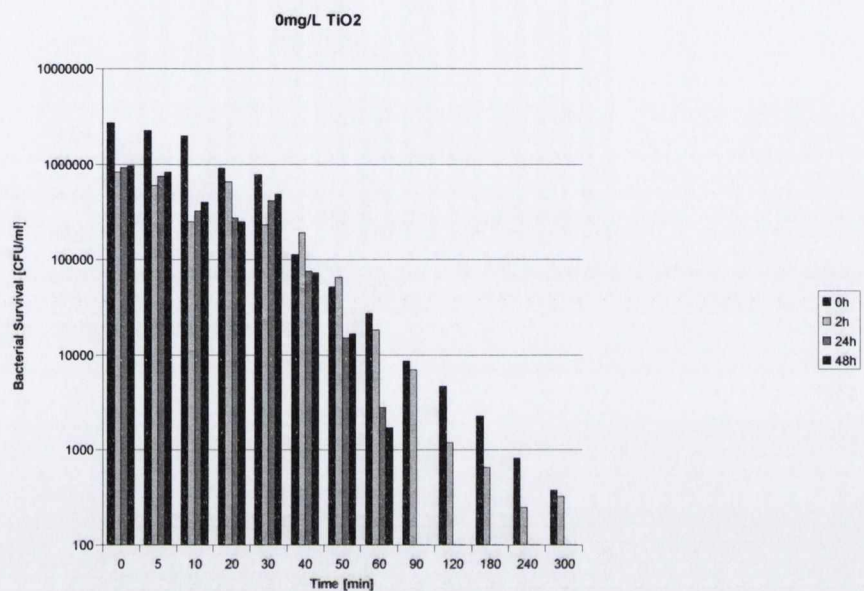
4.3.3.2 Post-irradiation events after solar treatment

The post-irradiation experiments conducted under natural sunlight show similar results as those conducted under UV light [Figures 4.3 (c) - (h)]. In the absence of photocatalyst, none of the samples plated two hours after testing dropped down to the MDL, while bacteria were still present 48 hours post-irradiation for samples irradiated for up to and including 360 minutes (Trial 1), 300 minutes (Trial 2) and 180 minutes (Trial 3). In stark contrast to this, when catalyst was used in the experiments the only 48 hour samples that showed any bacterial survival were the 0 minute samples, i.e. those before any photocatalysis took place.

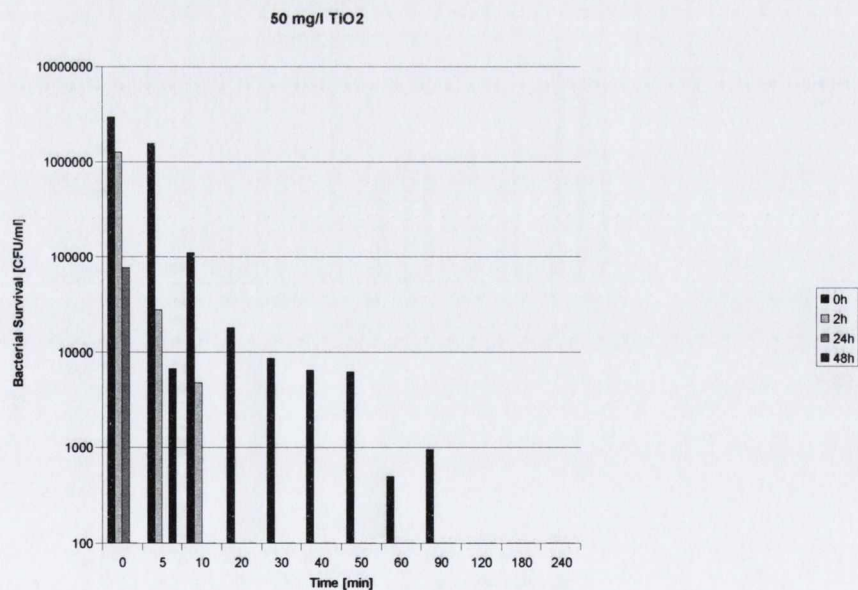
From the above results it can be inferred that photocatalytic treatment has a far more pronounced 'residual' bactericidal effect, as a dramatic decay in bacterial concentrations is seen after treatment has stopped. This is even more apparent when compared with the results of the experiments conducted in the dark [Figures 4.3 (i) and (j)]. In the dark experiments, bacterial counts remained almost constant, both during the reactor run and in the subsequent 48 h period in the dark. It is also noticeable that less post-trial die-off was seen in the presence of a catalyst for the control experiments carried out in the dark, a finding which accentuates the post-trial advantage of photocatalysis over photolysis in the trials conducted under light. A comprehensive list of hypotheses as to why a residual effect is seen in such experiments has been presented recently. These include the fact that some enzymes responsible for the defence of bacteria against oxidative stress may continue in the dark; the altered membrane permeability may allow hydrogen peroxide and cations to enter into the cells, increasing the perturbation of the redox reactions in the cell, a process that may continue in the dark; and the other by-products of photocatalysis may continue to attack cells in the dark (Rincón and Pulgarin, 2007).

It is interesting to note that the UV light alone [Figure 4.3(a)] seemed to have a more pronounced effect on post-irradiation events than sunlight [Figure 4.3(c)], despite the average

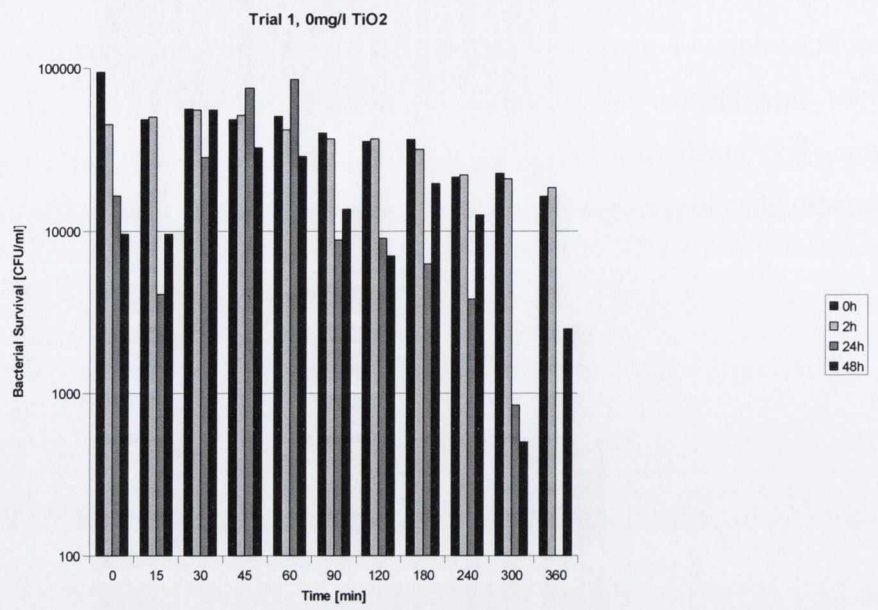
intensity being lower for the lamp than in each of the three trials. Again, as discussed earlier, this could be due to a number of factors such as UV fluctuation and other seasonal factors in the solar experiments. For example, the amount of cloud cover and position of the sun determine how much scattering of solar radiation occurs and shorter wavelengths are scattered more readily than longer wavelengths.



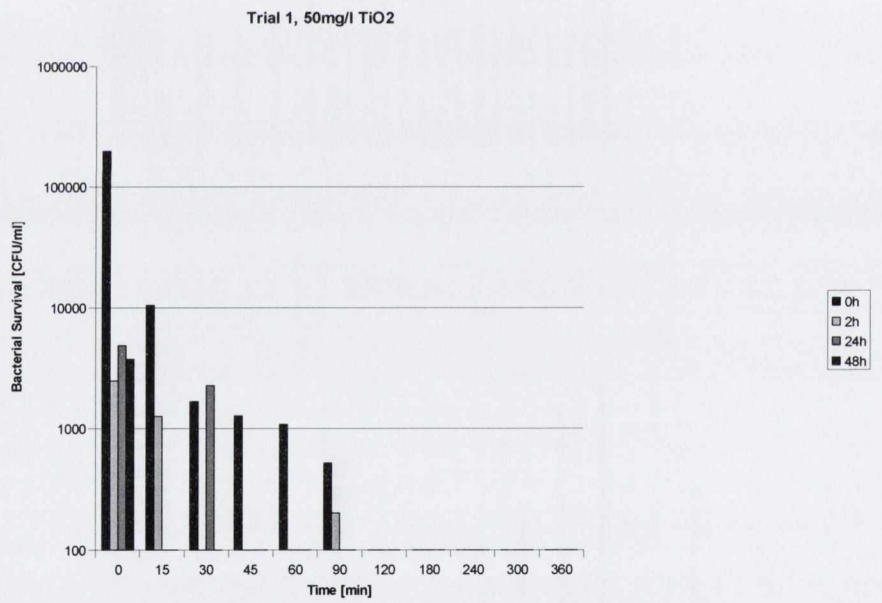
(a)



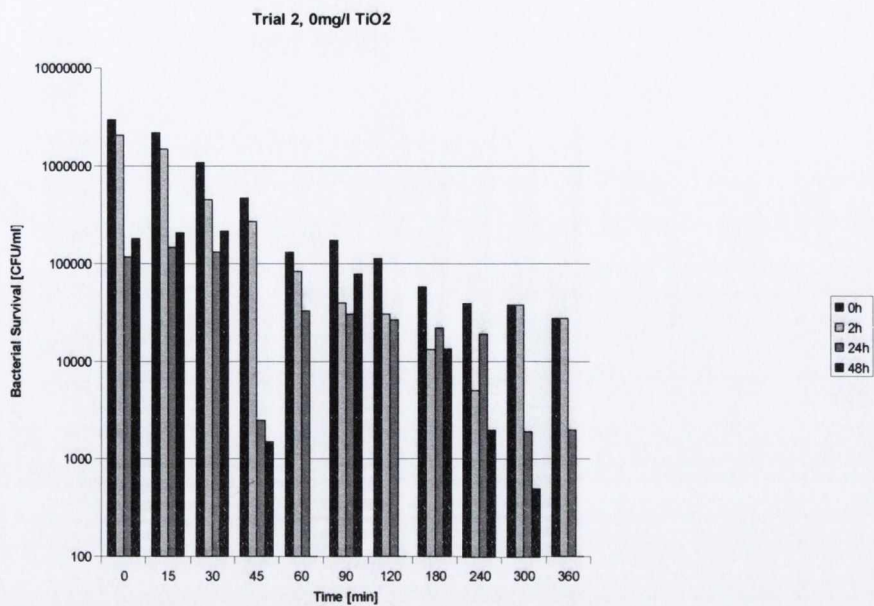
(b)



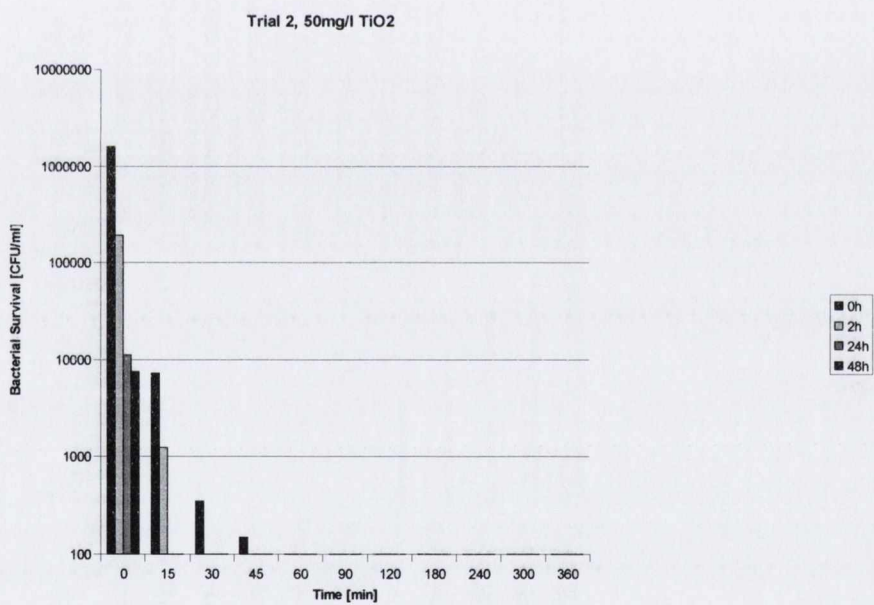
(c)



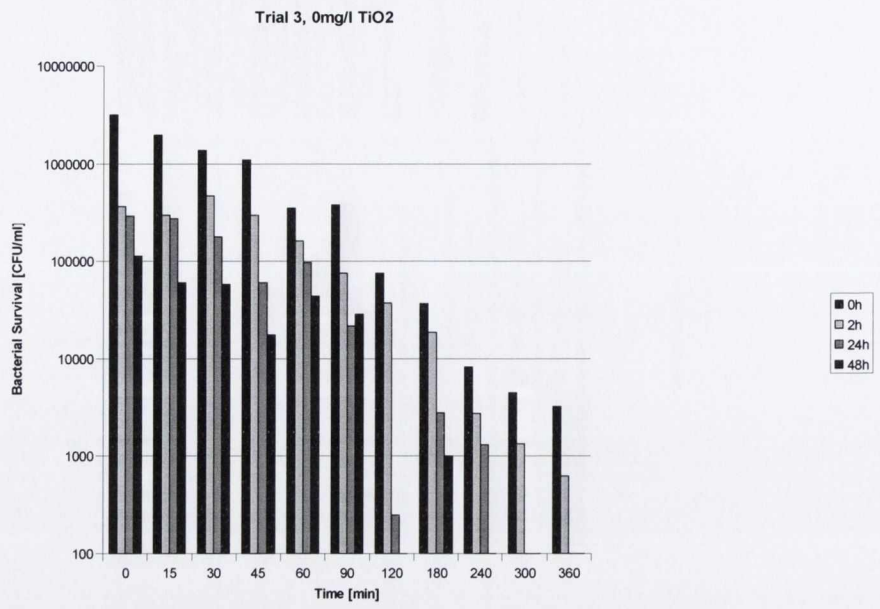
(d)



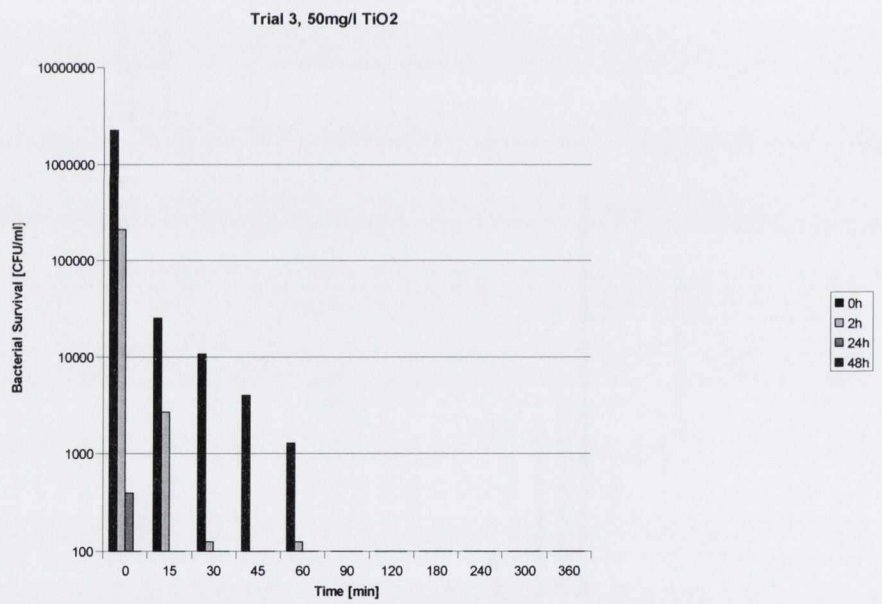
(e)



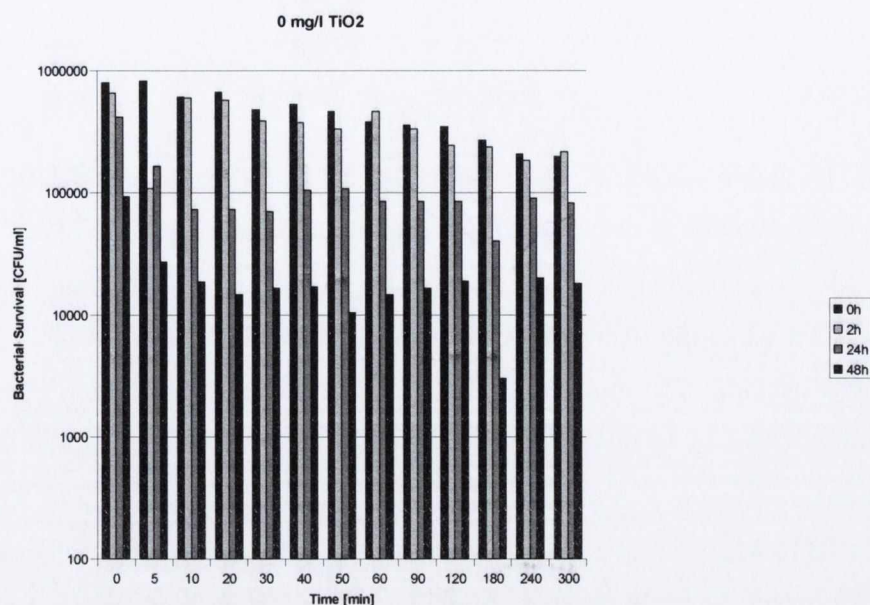
(f)



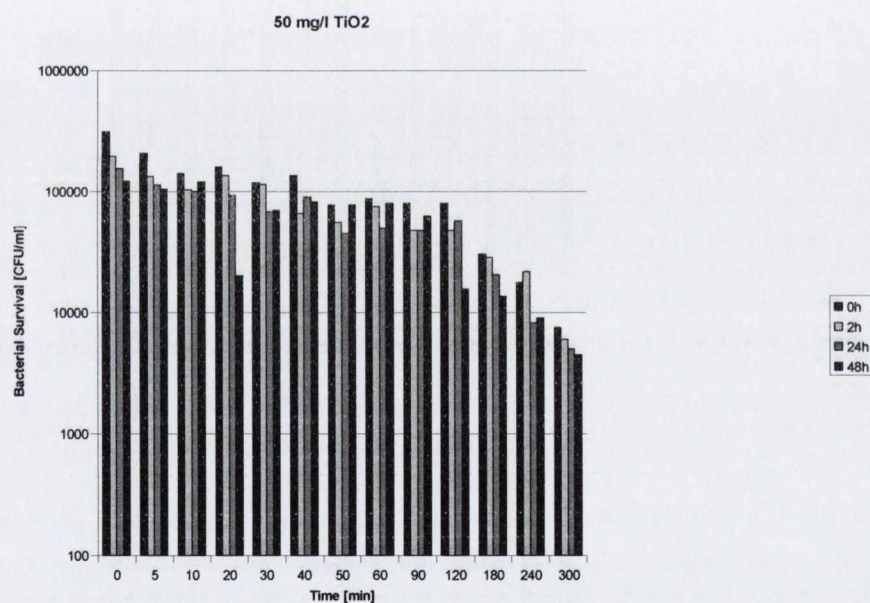
(g)



(h)



(i)



(j)

Figure 4.3 Bacteria numbers for each consecutive sample for time between sampling and plating of 2, 24 and 48 h using: (a) no TiO₂ and (b) 50 mg/L suspended TiO₂ under UV lamp; (c) no TiO₂ and (d) 50 mg/L suspended TiO₂ in Trial 1 under sunlight; (e) no TiO₂ and (f) 50 mg/L suspended TiO₂ in Trial 2 under sunlight; (g) no TiO₂ and (h) 50 mg/L suspended TiO₂ in Trial 3 under sunlight; (i) no TiO₂ and (j) 50 mg/L TiO₂ in the dark.

4.3.3.3 Effective Disinfection Time

It has been found that damage caused to cells can continue in the dark via the action of relatively stable oxidants which are generated during photocatalytic oxidation such as hydrogen peroxide, as discussed by Huang and coworkers (Huang *et al.*, 2000). This shows a clear advantage of photocatalysis over photolytic disinfection, as complete termination of cells is achieved when the treated water is stored for 24 hours because of post-irradiation die-off. This has encouraging implications, as it shows that post-irradiation bacterial die-off can be greatly accelerated in the presence of a photocatalyst. Similar results were found by other researchers (Rincón & Pulgarin, 2004b) in their investigations into Effective Disinfection Time (EDT) which is defined as the treatment time necessary to avoid some bacterial regrowth after 24 (or 48) hours in the dark, after irradiation has stopped. When TiO_2 was absent in this research, the EDT was not reached in the experimental time for all of the experiments. It must be noted that previous authors have found that a high enough dose of solar irradiation can, in fact, have a gradual detrimental effect on cell culturability post-irradiation (Berney *et al.*, 2006a). The amount of time needed to reach the EDT, however, may not be practical for the purposes of reactor systems.

In the presence of TiO_2 , the EDT was reached in all experiments undertaken, the EDT values being 10 minutes, 45 minutes, 15 minutes and 15 minutes [Figures 4.3 (b), (d), (f) and (h), respectively]. In the absence of TiO_2 , the EDT was significantly larger or not reached at all and the values in this case were 90 minutes, not reached, not reached and 300 minutes [Figures 4.3 (a), (c), (e) and (g), respectively]. The values obtained when TiO_2 was present are lower than the EDTs reported in previous papers (Rincón & Pulgarin, 2004b, 2007), however it is difficult to compare such experiments, as parameters such as the growth state of the bacteria are thought to have a significant effect on sensitivity to UV disinfection and post-irradiation characteristics, while the reactor characteristics and intensity of irradiation were also different. The same authors also found that regrowth/reactivation of bacteria occurred after photolytic treatment of bacteria, even when a bacterial concentration < 1 CFU/mL had been obtained. However, to verify the complete inactivation of *E. coli* in the presence of suspended TiO_2 , post-treatment samples need to be incubated in ideal growth conditions (Rincón & Pulgarin 2004a).

It was previously concluded from similar post-irradiation experiments to those undertaken in this chapter that solar TiO_2 treatment has a bactericidal effect, causing irreversible damage in cellular functions, while solar treatment alone merely has a bacteriostatic effect (Gelover *et al.*, 2006). It must be reiterated, though, that high enough doses of solar UVA have been shown to cause sufficient membrane damage to prevent repair mechanisms from happening, meaning that SODIS can in some cases deliver a lethal dose (Berney *et al.*, 2006a). The bactericidal effect of photocatalysis post-irradiation is a particularly attractive characteristic for continuous flow reactor design where the disinfected water is likely to be stored in a closed reservoir for some time before consumption.

4.3.3.4 The use of different types of water

The direct comparison between the experiments detailed in Section 4.2.3 is difficult to achieve, as each experiment was carried out on a different day. Weather conditions varied from very sunny to completely overcast, with large variations in solar intensity during experiments. To this end, the EDT_{48} obtained from each test, taken from Figure 4.4, is presented in Table 4.4, along with the cumulative UV dose required to reach the EDT and the peak UV value received prior to the EDT. It is interesting that in the case of the distilled water, the EDT_{48} was reached earlier in the absence of TiO_2 , despite the cumulative doses received being lower in both trials than in the two photocatalytic tests. It would be expected that of the photolytic tests, the EDTs in distilled water would be lowest, as the lack of ions and nutrients in distilled water is thought to apply osmotic and starvation stresses upon bacteria, thus rendering them more susceptible to disinfection (Sichel *et al.*, 2007, Berney *et al.*, 2006). The higher EDT_{48} (and EDT_{24}) values found with TiO_2 in distilled water are surprising, considering earlier findings in this chapter as well as the fact that disinfection rates were higher in distilled water when catalyst was present.

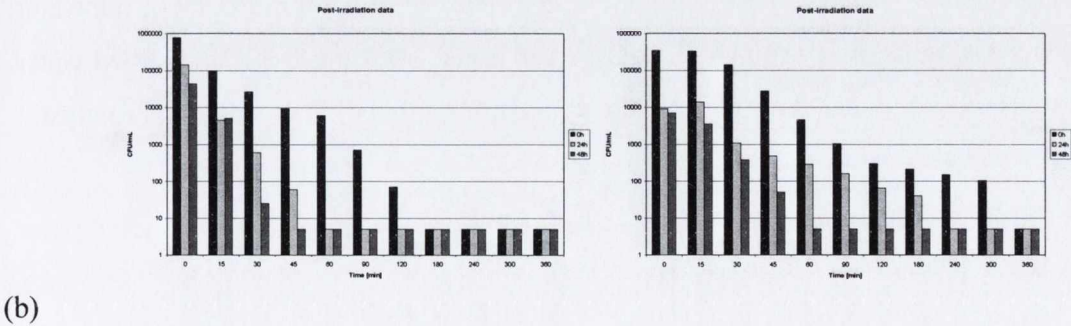
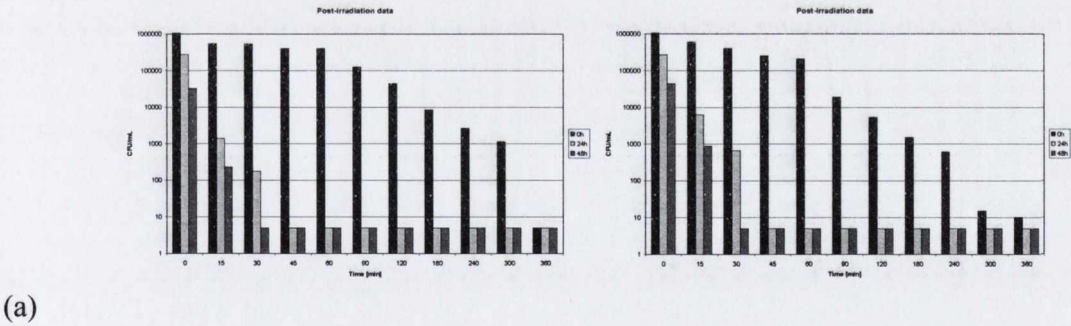
In rainwater, the EDTs were the same (60 min) with and without catalyst on cloudy days, while photocatalysis showed a slight advantage on sunny days, with the EDT reached after 30 minutes, or 2.32 kJ/L, compared to 45 minutes (3.55 kJ/L) in the absence of catalyst. In general, the EDT_{48} was higher in rainwater than in distilled water, which could be explained by the expected presence of ions and nutrients in rainwater, which would provide a more conducive environment for bacterial growth.

River water showed the most interesting findings in terms of post-irradiation events, as regrowth was witnessed for the first time. As can be seen in Figure 4.4 (e), there was an increase in bacterial numbers in the dark when TiO_2 was not present. In the first trial, the MDL was reached after 300 minutes, yet regrowth still occurred in both the 300 and 360 min samples. It would appear unusual that regrowth is more pronounced in samples later on in the experiment, and that no multiplication of bacteria occurs in the sample taken at the very start. Ideal growth conditions are present, with a temperature of 37 °C and, in the case of the river water, there is probably a plentiful supply of nutrients. This anomaly could be explained by the fact that bacterial growth is substrate limited and that when samples enter the incubator with a high concentration of bacteria, there is not enough substrate for all the cells and so some decay occurs. In the case of samples where the bacterial concentration is low, i.e. those taken towards the end of experiments, the substrate to bacteria concentration is high and provides an ideal environment for growth of bacteria. When TiO_2 was introduced [Figure 4.4 (f)], no regrowth was observed, and the EDT_{48} was reached after 45 and 90 min in the two trials. Again, if the dose received until EDT values are investigated, these were higher for river water than for both distilled water and rain water, which can be attributed to possibly higher levels of nutrients and ions and possible existing biological contamination.

The higher treatment time and UV dose required to permanently inactivate *E. coli* in photocatalytic river water trials than in distilled in rain water, as well as the presence of regrowth in photolytic river water trials shows that the type of water has a significant influence on results found in solar disinfection studies. Care must be taken when drawing conclusions from work on distilled water, as results may not be directly applicable to practical applications. The osmotic and starvation stresses suffered by cells in the absence of ions and nutrients render bacteria more susceptible to disinfection and less able to activate repair mechanisms in the dark.

Water Type	TiO ₂ concentration [mg/L]	EDT ₄₈ [min]	Cumulative dose received until EDT ₄₈ [kJ/L]	Peak UV received prior to EDT ₄₈ [W/m ²]
Distilled	0	30	0.42	4.1
Distilled	0	30	0.75	8.9
Distilled	50	45	3.5	36.9
Distilled	50	60	1.29	8.0
Rainwater	0	45	3.55	32.1
Rainwater	0	60	1.39	8.6
Rainwater	50	60	1.85	10.2
Rainwater	50	30	2.32	27.2
River water	0	Not reached	>15.9	32.7
River water	0	Not reached	>8.1	30.2
River water	50	45	9.6	30.7
River water	50	90	6.45	12.3

Table 4.4 A comparison of the EDT₄₈ for experiments conducted in different water types



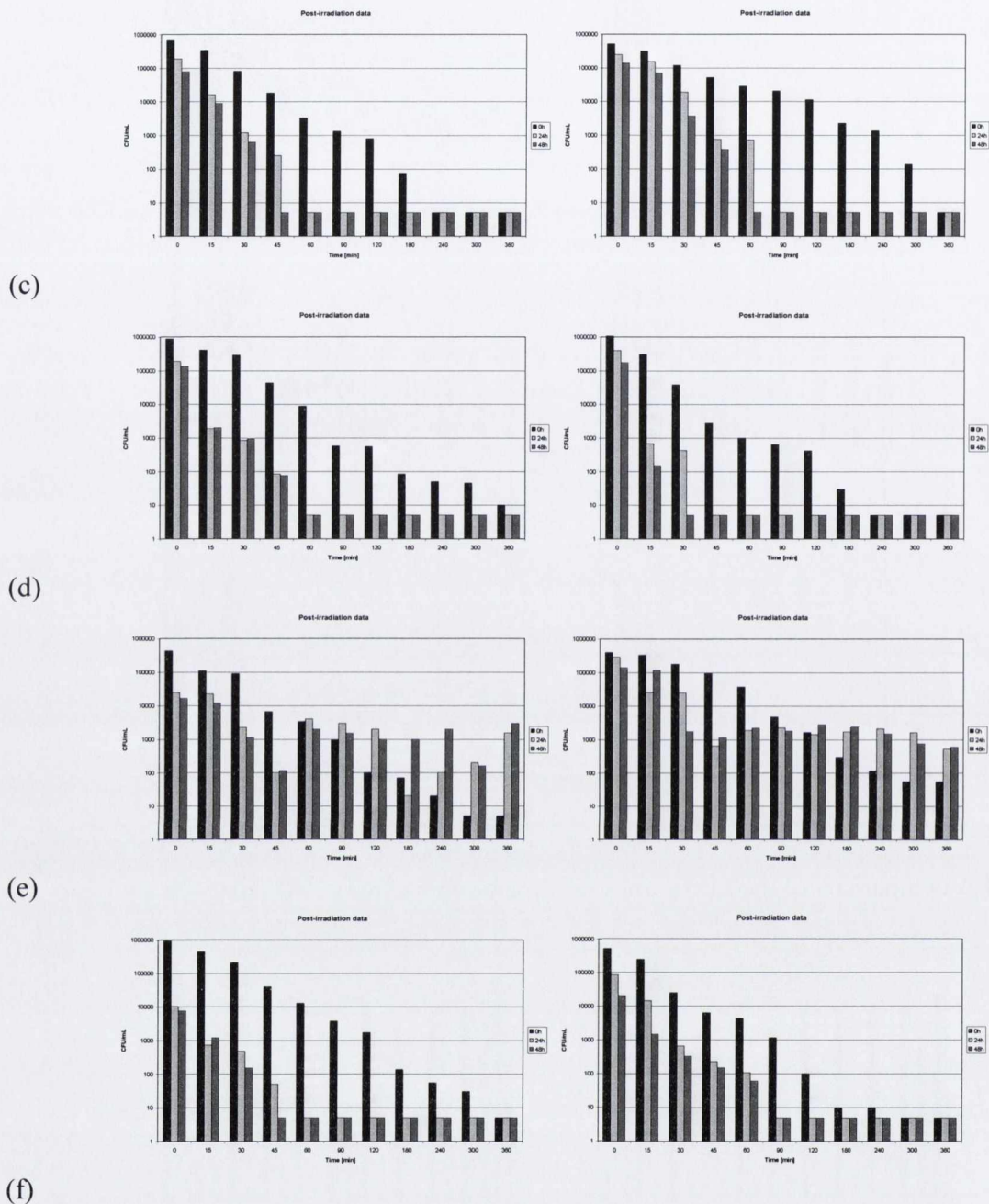


Figure 4.4 Post irradiation events for experiments conducted with different types of water. (a) 0 mg/L TiO_2 , distilled water; (b) 50 mg/L TiO_2 , distilled water; (c) 0 mg/L TiO_2 , rainwater; (d) 50 mg/L TiO_2 , rainwater; (e) 0 mg/L TiO_2 , river water; (f) 0 mg/L TiO_2 , distilled water. Note: two trials were conducted on separate days for each combination of water and catalyst.

4.3.3.5 Transmission Electron Microscopy

A sample of the images taken from the electron microscope work are presented in Figure 4.5. The photolytic and photocatalytic samples exhibited 4.2-log and 4.8-log disinfection, respectively, after 15 h of illumination, which are extremely low values in relation to what would be expected from inactivation kinetics seen earlier in this chapter. It must be remembered, however, that the initial bacterial concentrations were 10^3 times higher than those used in the CPC, meaning the turbidity was increased, while the products of cell lysis would mean that there would also have been more organic matter available to surviving *E. coli*. Figure 4.5 (a) shows cells after 15 h of illumination without catalyst, while Figures 4.5 (b) and (c) show cells before and after 15 h of photocatalytic treatment. The TiO_2 particles can be seen clearly and, as expected, are much smaller than the *E. coli* cells. It would appear from Figure 4.5 (a) that there was some degree of separation of the lipid bilayer after 15 h of illumination in the absence of catalyst, but there are no signs of significant membrane damage. Figure 4.5 (b) shows the cells and catalyst particles before illumination and again, in the picture on the right, there is evidence of stressed cells. This could be due to the osmotic and starvation stresses applied by the distilled water, as discussed previously in Section 4.3.3.4. Although some effect would be expected, the agglomeration of catalyst seems exaggerated, but it must be remembered that samples were centrifuged and redistributed in agarose during the microscopy preparation, so the large degree of agglomeration could have occurred at this stage. Examining Figure 4.5 (c), it can be seen that cells are still very much intact after 15 h of photocatalytic treatment, with no strong evidence of the membrane damage which would be expected, and has been seen previously in atomic force microscopy (AFM) studies on the photocatalytic disinfection of *E. coli* (Sunada *et al.*, 2003). It would appear that more separation of the external cell layers occurs in the vicinity of TiO_2 attachment to the cells, but as this effect has been seen in the photolytic experiments and before illumination, no definite conclusions can be drawn.

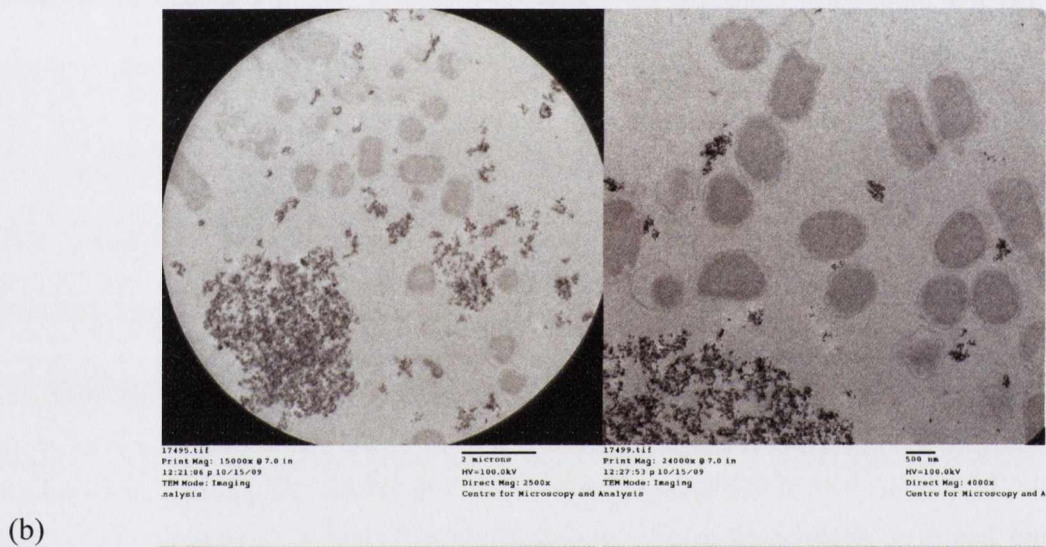
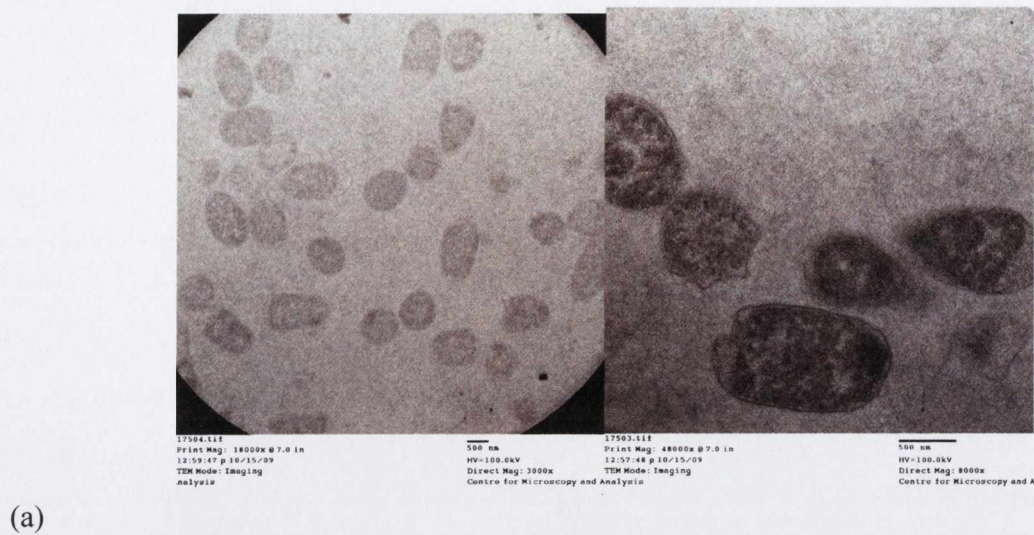


Figure 4.5 TEM images of bacteria (a) after 15 h illumination - no TiO_2 ; (b) before treatment - 50 mg/L TiO_2 ; and (c) after 15 h illumination - 50 mg/L TiO_2 .

5. STROBOSCOPIC LIGHT EXPERIMENTS

5.1 Introduction

In this chapter, an investigation was carried out into the effect of introducing a variety of illumination interruption patterns on the disinfection of *E. coli* in the bench-scale CPC (detailed in Section 3.1.4.1).

Previous research into this phenomenon has yielded quite conflicting results. In a photolytic experiment using three CPC collectors in series, it was found that when two of the reactors were covered, disinfection proceeded as well as it had with the three reactors open. This apparent anomaly led the authors to consider a number of factors which might have been influential in the results, such as mechanical stress, contamination from previous experiments in the reactor or a temperature related mechanism. These were all discounted and the authors instead hypothesised that it could be due to a stroboscopic shock mechanism caused by the bacteria traveling between areas of light and dark for different periods of time (McLoughlin *et al.*, 2004a). Fernández *et al.* (2005) performed similar experiments in which CPC tubes were covered up to give illuminated areas of 0.125 m², 0.25 m², 0.5 m² and 0.75 m². They found that disinfection proceeded at the same rate with respect to time in all four experiments, and carried out further experiments to investigate why this may be the case. They concluded that there must be a synergistic effect between the illuminated area and experimental runtime, due to mechanical stresses imposed on bacteria by the flow rate.

It has been previously concluded in a paper that interruption of illumination during photocatalytic treatment aids bacterial survival, especially if interruption occurs near the beginning of illumination (Rincón & Pulgarin, 2003). The effect was attributed to bacterial defense mechanisms in the dark being more prevalent, whereby the synthesis of superoxide dismutase (SOD) enzymes catalyses the conversion of superoxide and hydroxide radicals into hydrogen peroxide and oxygen. Thus, the concentration of highly oxidizing •OH radicals is decreased. It was also noted that interrupted illumination has a different effect on various organisms and the results found in their research on *Escherichia coli* was not reflected in all the literature. In another paper, *Bacterioides fragilis* was also seen to show more resistance under an interrupted illumination regime, while the MS2 bacteriophage was seen to be more

susceptible to such treatment (Laot *et al.*, 1999). *B. pumilis* spores were also found to be treated more readily under interrupted illumination (Pham *et al.*, 1995).

Elsewhere, it has been seen that for solar photocatalytic systems, once a threshold UV dose is received, disinfection is achieved independent of the irradiated area. In the absence of a catalyst, kinetics were found to depend more on irradiation and the threshold dose was found to be higher (Sichel *et al.*, 2007b).

5.2. Materials and Methods

Again, all experiments were conducted using *Escherichia coli* K-12 (ATCC 10798), which was propagated and enumerated as set out in Section 3.2.1. 1 L of sterile water was used in each experiment, with photocatalyst samples prepared according to Section 3.1.1. 50 mg/L of catalyst was used, based on findings in Section 4.3.1. The bench-scale CPC (Section 3.1.4.1) was used in all experiments and was prepared as detailed in Section 3.2.3.1. 1×10^6 CFU/mL of bacteria was dosed into the reservoir, with samples taken at 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 minutes. Post-irradiation tests were carried out on all samples, as discussed in Section 3.2.3.1. A series of experiments was conducted, exposing the water in the reactor to different illumination regimes. Black cardboard was cut into strips of equal width and placed over the reactor tubes in configurations shown in Figure 2.1. The configuration characteristics are summarized numerically in Table 5.1. Each experiment was undertaken three times to ensure the reproducibility and accuracy of the results. Dark control experiments were run in the presence and absence of TiO_2 to investigate the effect of mechanical stress on the bacteria, as it has been reported previously that a large proportion of bacteria die-off in CPC systems is due to mechanical stress (Sichel *et al.*, 2007a). The reactor tubes were covered using pieces of black cardboard to generate the desired patterns of dark/light. Results were presented as plots of $\log_{10}(\text{bacterial survival})$ vs time and cumulative dose, which was calculated using Eqn. 3.6.

Configuration	Number of Openings	Time spent under illumination per opening [s]	Equivalent number of pipes under illumination	TiO ₂ concentration [mg/L]	Time under illumination per traverse [s]	Flash frequency [flashes/min]	Time under illumination [%]	EDT ₂₄ [min]
1	3	0.388	3	0 & 50	1.164	8.4	5.4	180(photolytic), 15(photocatalytic)
2	1	0.388	1	0 & 50	0.388	2.8	1.8	>420 (photolytic), 45(photocatalytic)
3	1	0.194	0.5	50	0.194	2.8	0.9	60
4	12	0.032	1	50	0.388	33.6	1.8	30
5	18	0.022	1	50	0.388	50.5	1.8	15
6	6	0.065	1	50	0.388	16.8	1.8	45
7	18	0.065	3	0	1.164	50.5	5.4	>420
8	6	0.194	3	0	1.164	16.8	5.4	180
9	12	0.097	3	0	1.164	33.6	5.4	>420
10	6	0.388	6	0 & 50	2.328	16.8	10.9	180(photolytic), 30(photocatalytic)
11	42	0.028	3	0	1.164	117.8	5.4	360

Table 5.1 Comparison of the illumination regimes

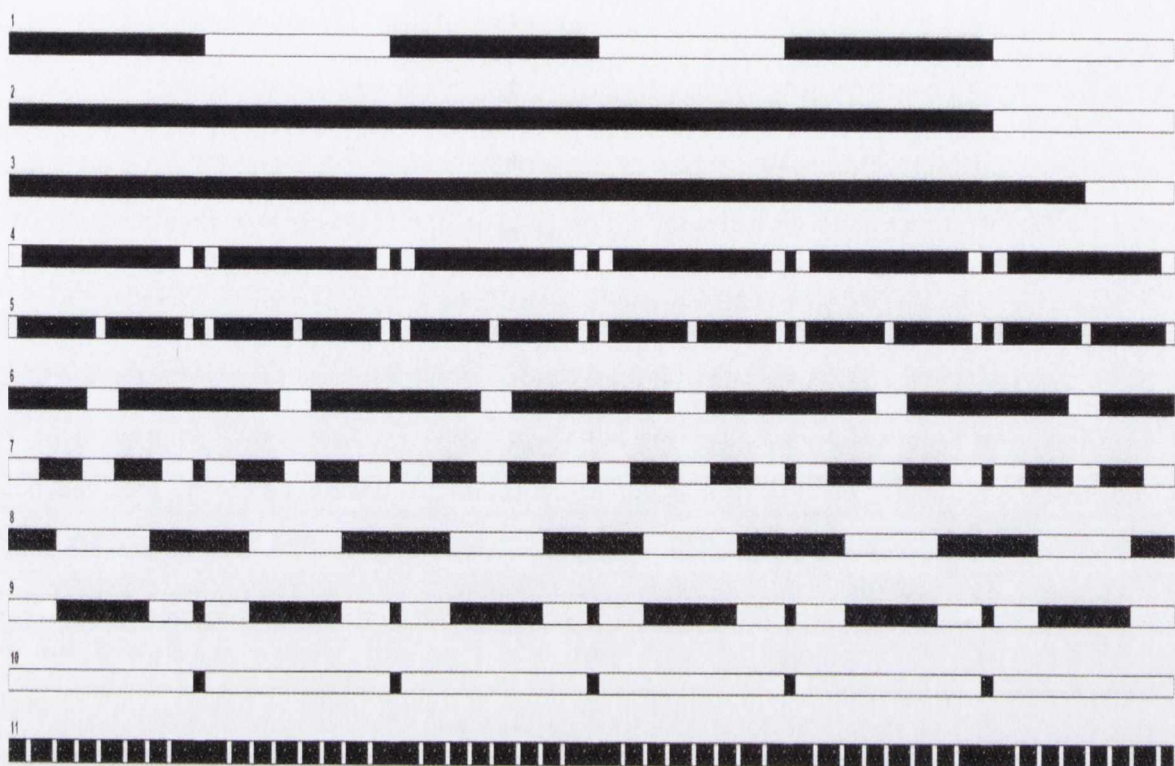


Figure 5.1 Illumination regimes. Note: the time spent in the reservoir and flowing to and from the reactor is not included in the above diagrams.

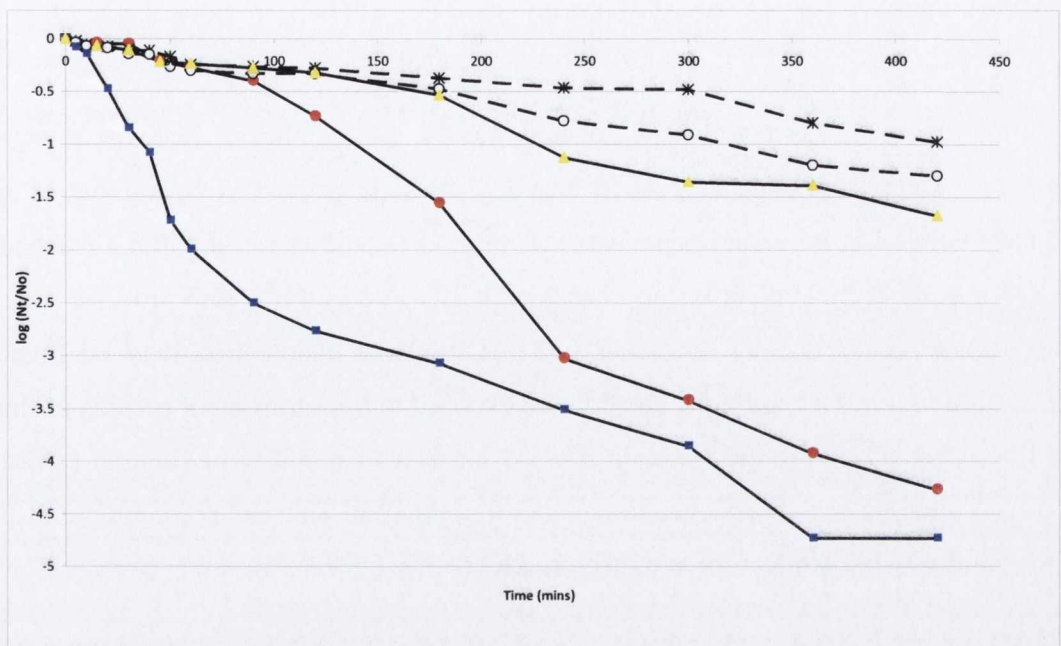
5.3. Results and discussion

5.3.1 Photolytic tests

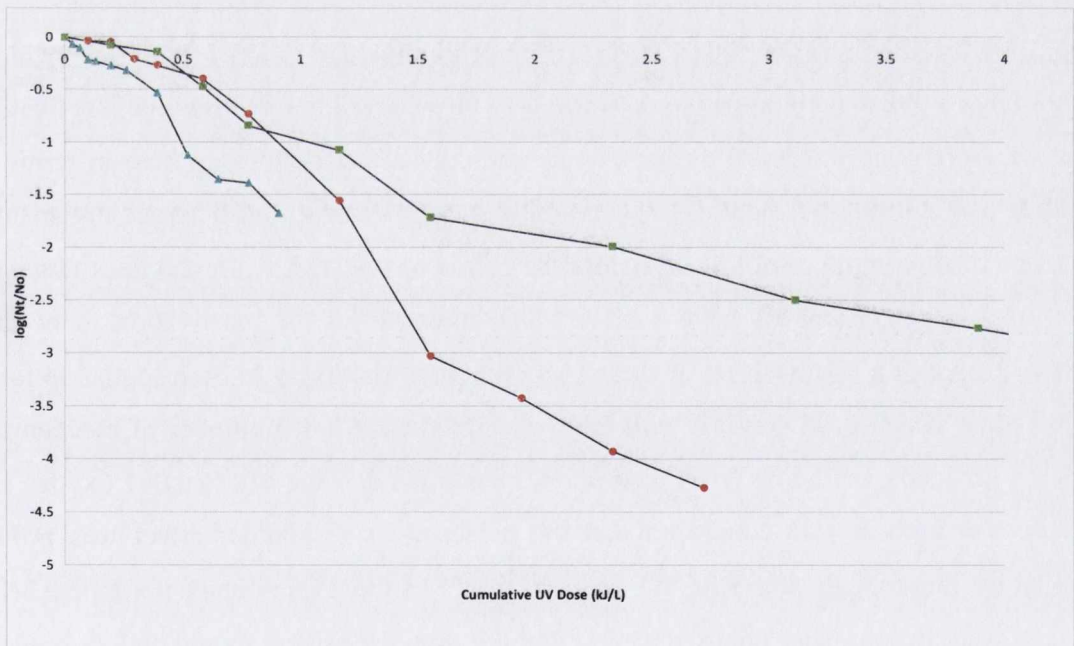
5.3.1.1 Disinfection rates with increased illuminated area

In the photolytic tests, as the net illuminated area was increased, there was a marked improvement in the rate of disinfection with respect to time, as can be seen in Figure 5.2 (a). Configuration 2, in which one pipe was left open, showed only marginal improvement from the dark experiments, suggesting that perhaps a minimum dose needs to be received before effective disinfection can proceed. The disinfection rates when plotted against time are in stark contrast to the rates when plotted against cumulative dose, as shown in Figure 5.2 (b). Per unit UV received, the configuration with just one pipe left uncovered showed the best performance, which would suggest that disinfection does not improve linearly with respect to cumulative dose received. Instead it appears to be linked to a combination of dose and illumination time. It has been suggested elsewhere (Rincón & Pulgarin, 2004b) that cumulative dose is not a suitable parameter for standardising photolytic disinfection. Bacteria in configuration 2 had to complete six circuits of the reactor to receive the same cumulative dose encountered by the configuration 10 bacteria in one circuit. It would seem intuitive, therefore, that other stresses encountered during that time period would account for the improved disinfection with respect to cumulative dose.

Previous results have shown, however, that when the illuminated area is reduced disinfection rates with respect to time (as opposed to dose) can be just as high, and sometimes higher (McLoughlin *et al.*, 2004a, Fernández *et al.* 2005). This would in turn mean that the quantum efficiency of the regimes receiving more irradiation is far lower. In the latter paper, the authors went on to hypothesise, after finding that bacterial inactivation increased with higher flow rates, that one factor could be the increased capability of bacteria to adapt to stresses such as incoming photons with increased residence time. So, the decline in inactivation rate with increased residence time under illumination per traverse reported elsewhere could be explained by the adaptation of bacteria to the effects of UV.



(a) ✱ Dark experiment, 0 mg/L TiO₂; ○ Dark experiment, 50 mg/L TiO₂; ▲ 1 pipe open (config. 2); ● 3 pipes open (config. 1); ■ 6 pipes open (config. 10).



(b) ▲ 1 pipe open (config. 2); ● 3 pipes open (config. 1); ■ 6 pipes open (config. 10).

Figure 5.2 Plot of $\log(N_t/N_0)$ against (a) time and (b) cumulative UV dose for 0 mg/L TiO₂.

5.3.1.2 Disinfection rates for different illumination patterns with area kept constant

When the illuminated area was kept constant, but the illumination regime was changed, very little effect was seen (Figure 5.3). There was a slight improvement as the number of openings was reduced, and in no experiments was the Method Detection Limit (MDL) reached within the 420 min. Photolytic disinfection occurs primarily due to a physical process, which takes place over a relatively long timescale and this mode of disinfection may be favoured by illumination regimes whereby the bacteria are exposed to light for longer periods of time. This could be balanced by the previously discussed increased capability of bacteria to respond to photolysis with increased residence time under illumination. This balance could be responsible for the similarity of disinfection rates seen in Figure 5.3.

Again as alluded to in Section 4.3.2, conditions for photolysis in the small-scale CPC are far from ideal, as the time spent in the dark per traverse is relatively high even before the tubes are partially covered. Therefore the results above are not comparisons of continuous and interrupted illumination, but of variations in the already small irradiated volume.

Very little inactivation occurred in the first hour of illumination in any of the trials, which may suggest that a threshold cumulative dose needs to be achieved before effective inactivation can proceed. Berney *et al.* (2006) discussed the ‘shoulder effect’ witnessed here in terms of the multihit and multitarget theories. The multihit scenario states that a target organism must receive a distinct number of hits, n , before the cell is inactivated, while the multitarget theory states that target organisms have n sites which must be hit for inactivation to occur. The authors proposed a combination of these two scenarios was likely to be responsible for UVA disinfection, because, in contrast with UVC disinfection, it has a number of mechanisms by which it proceeds, including DNA disruption, membrane damage and reactive oxygen species effects. The authors also concluded that the presence of a shoulder effect was influenced heavily by the growth phase of the bacteria used. In the experiments presented here, the bacteria were in stationary phase, meaning that the shoulder effects should be less pronounced than they would be for fast-growing cells.

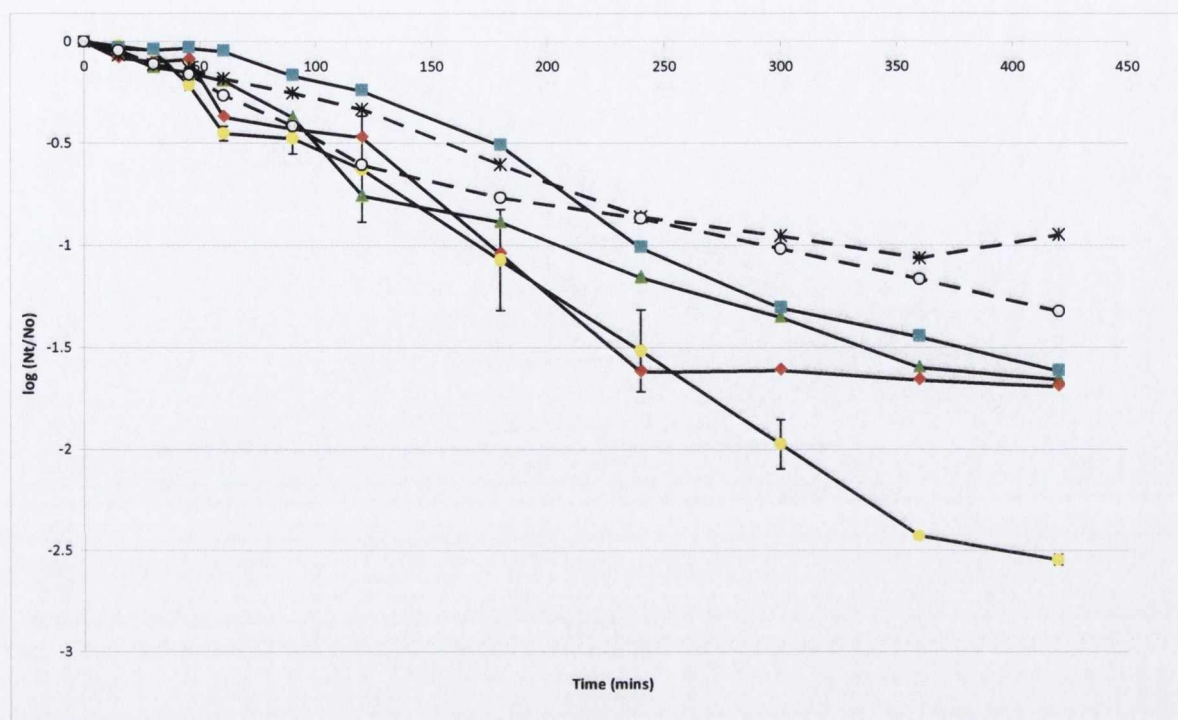
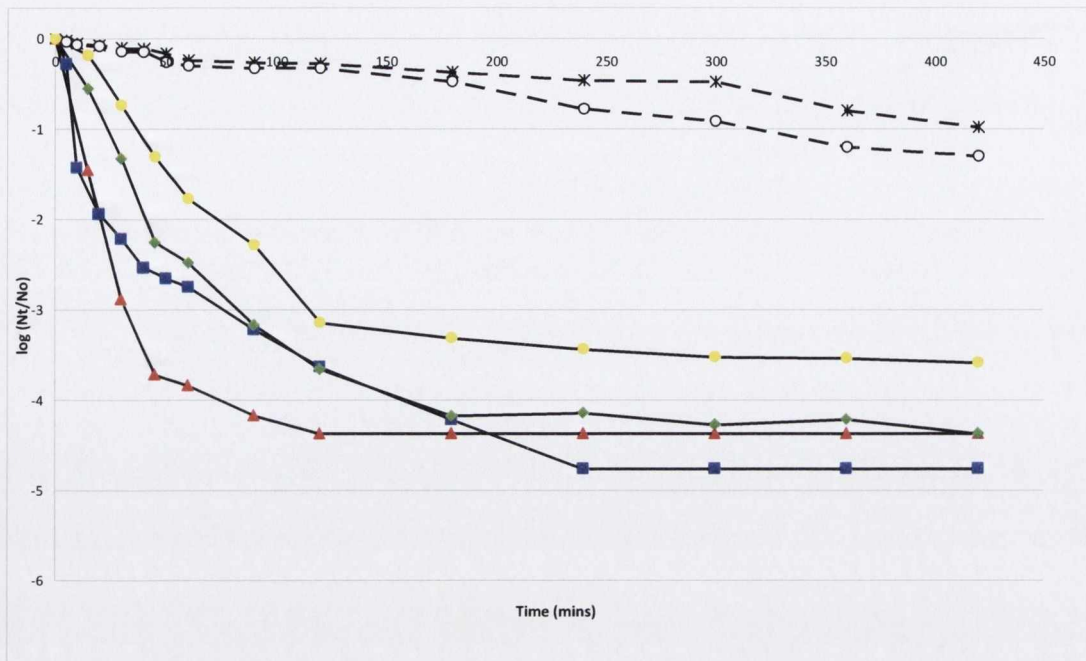


Figure 5.3 Comparison of disinfection rates for different numbers of openings, with constant effective illuminated area of 3 pipes. No photocatalyst present. [✱ Dark experiment, 0 mg/L TiO₂; ○ Dark experiment, 50 mg/L TiO₂; ● 6 openings (config. 8); ◆ 12 openings (config. 9); ▲ 18 openings (config. 7); ■ 42 openings (config. 11)]. Note: error bars are just on one line to avoid confusion.

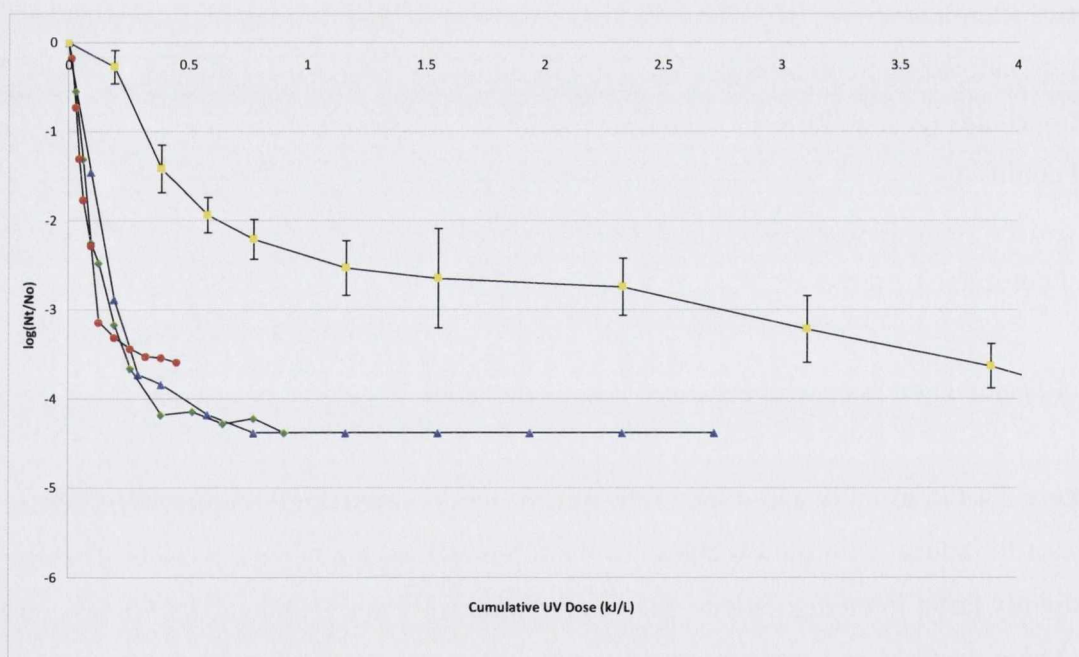
5.3.2 Photocatalytic tests

5.3.2.1 Disinfection rates with increased illuminated area

In Figure 5.4 (a), as more and more of the reactor was covered up, the initial disinfection rate was seen to reduce, with the exception of the three and six pipe configurations. Disinfection was slightly faster in configuration 1 than configuration 10, despite having half the illuminated area. These findings are very similar to previous results, where it was found that once a threshold intensity was received, disinfection occurred regardless of irradiated area (Sichel *et al.*, 2007b). The cumulative dose plot shows similar disinfection rates in relation to this parameter, with the exception of the 6 pipe illuminated area [Figure 5.4 (b)]. This configuration was much less efficient than the other three in terms of kill per dose received.



(a) ✱ Dark experiment, 0 mg/L TiO_2 ; ○ Dark experiment, 50 mg/L TiO_2 ; ● $\frac{1}{2}$ pipe open (config. 3); ◆ 1 pipe open (config. 2); ▲ 3 pipes open (config. 1); ■ 6 pipes open (config. 10).



(b) ● $\frac{1}{2}$ pipe open (config. 3); ◆ 1 pipe open (config. 2); ▲ 3 pipes open (config. 1); ■ 6 pipes open (config. 10). Note: error bars are just on one line to avoid confusion.

Figure 5.4 Plot of $\log(N_t/N_0)$ against (a) time and (b) cumulative UV dose for 50 mg/L TiO_2 .

5.3.2.2 Disinfection rates for different illumination patterns with area kept constant

The experiments comparing the number of openings were conducted with an effective illuminated area of one pipe, as opposed to three pipes as in the photolytic experiments. This was to ensure that disinfection occurred over a more observable timescale. With the illuminated volume kept constant, there was a significant difference in the disinfection rates among the various illumination patterns (Figure 5.5). With the exception of configuration 2, the disinfection rate increased with increased number of openings (i.e. higher flash frequency), which is in contrast to the findings from the photolytic experiments, where little effect was seen. In contrast to photolysis, indirect disinfection, involving the production of a large number of reactive oxygen species, is a photochemical process which occurs over a much shorter period of time and it is hypothesised that there may be a limiting concentration of reactive oxygen species (ROS) reached in the illuminated patches at any one time. By dividing the reactor into more illuminated areas, it may allow for the more efficient production and reaction of these species. This would explain why the photocatalytic disinfection was favoured by the increased number of pipe openings, while very little effect was seen in the photolytic tests. In previous work, interrupted illumination was found to be less efficient than continuous illumination during photocatalytic disinfection, as dark periods favoured the self defense mechanisms of the bacteria against reactive oxygen species. It was thought that the ratio between the time spent under illumination and in the dark, as well as the length of illumination time determines the hydroxyl radical production and thus the disinfection rate (Rincón & Pulgarin, 2003). Although this may seem to contradict the results presented here, the cited research investigated much longer periods of interruption, whereby illumination was stopped for 40 s at a time. In these experiments, a comparison was made, not between continuous and interrupted illumination, but between varying short periods of interruption. When the time spent in each dark and light patch was shorter, photocatalytic disinfection was favoured by having more, shorter patches of illumination. It is thought that as the time spent in each period of dark decreased, it became less favourable for self defense mechanisms against ROS to be activated. In the experiments presented in this section, as the residence time in each patch of light increased, so did the resistance to disinfection. The fact that configuration 2 yielded a better disinfection rate than configurations 4 and 6 could be related to a trade off between the direct and indirect mechanisms.

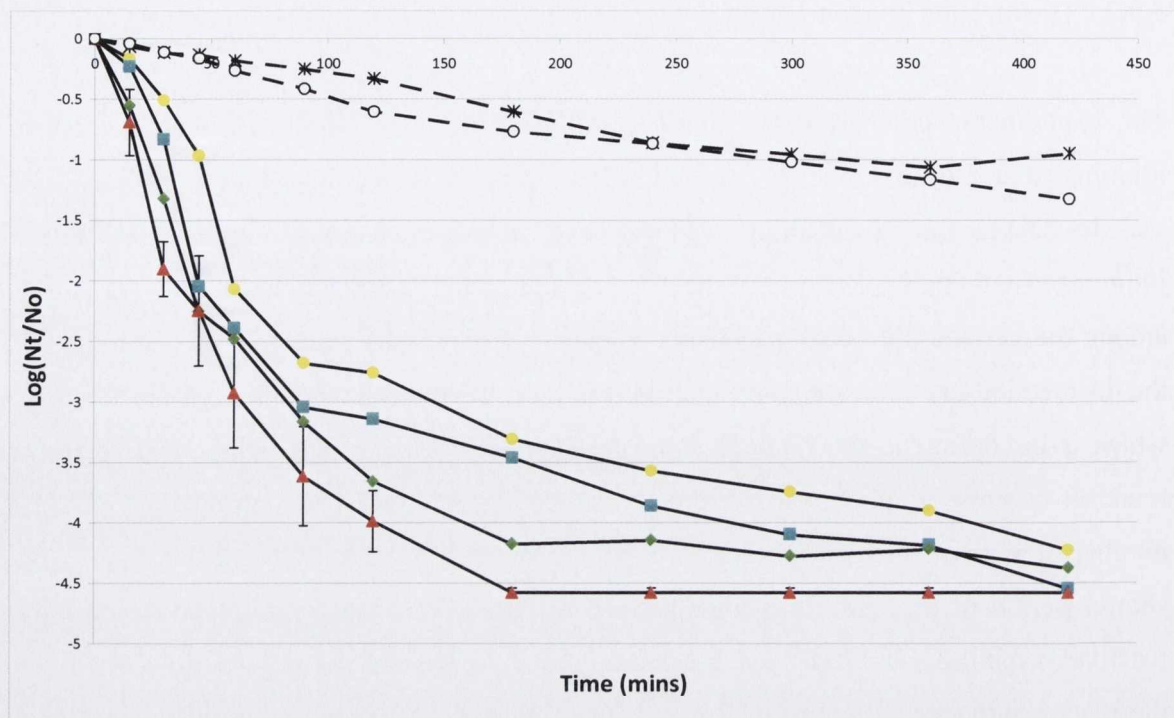


Figure 5.5 Comparison of disinfection rates for different numbers of openings, with constant effective illuminated area of 1 pipe. 50 mg/L photocatalyst present. [⌘ Dark experiment, 0 mg/L TiO₂; ○ Dark experiment, 50 mg/L TiO₂; ♦ 1 opening (config. 2); ● 6 openings (config. 6); ■ 12 openings (config. 4); ▲ 18 openings (config. 5)]. Note: error bars are just on one line to avoid confusion.

In order to put the disinfection rate achieved by configuration 5 into perspective, the disinfection rates from tests in which the illuminated area consisted of one pipe (configuration 5), three pipes and six pipes are compared in Figure 5.6. The MDL is reached after 120 min for the 3-pipe illuminated area, while the 1-pipe and 6-pipe illuminated areas reach the MDL after 180 min. The MDL was not reached when only half a pipe was left open. In other words, the most effective one-pipe configuration was as effective as the 6 pipe configuration. This has very interesting implications in reactor design, as it suggests that, once the threshold dose is received, a smaller UV dose can cause disinfection rates just as high as those brought about by a much larger dose. This has been observed previously (Sichel *et al.*, 2007b). It is hoped that reactor systems such as those outlined in Section 2.7.4.1 could be reduced in size and lead to more efficient treatment of drinking water.

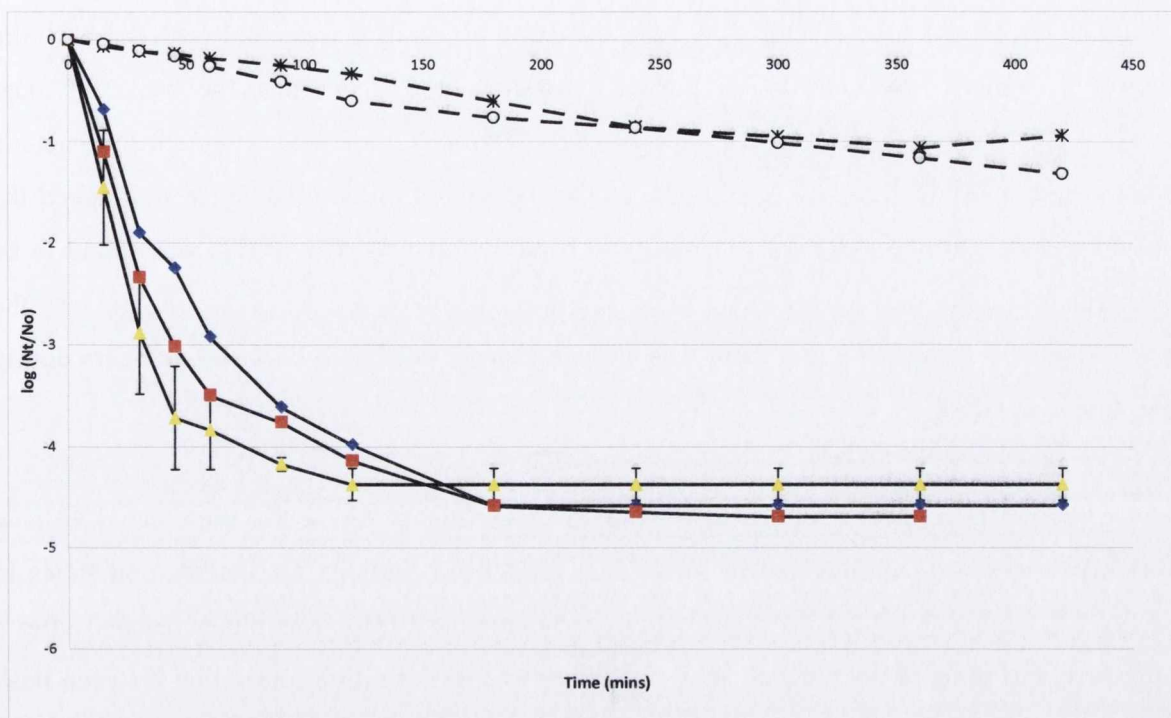


Figure 5.6 Comparison of optimal 1 pipe configuration with 3 and 6 pipe configurations. [X Dark experiment, 0 mg/L TiO₂; ○ Dark experiment, 50 mg/L TiO₂; ◆ 1 pipe area (config. 5); ▲ 3 pipe area (config. 1); ■ 6 pipe area (config. 10)]. Note: error bars are just on one line to avoid confusion.

5.3.3 Post-irradiation activity

As reported in Chapter 4, Effective Disinfection Time has been suggested as an effective parameter by which to compare photolytic and photocatalytic experiments, and is defined as the illumination time required to cause total inactivation of bacteria, with no subsequent regrowth during a 24 or 48 h period in the dark (Rincón & Pulgarin, 2004a).

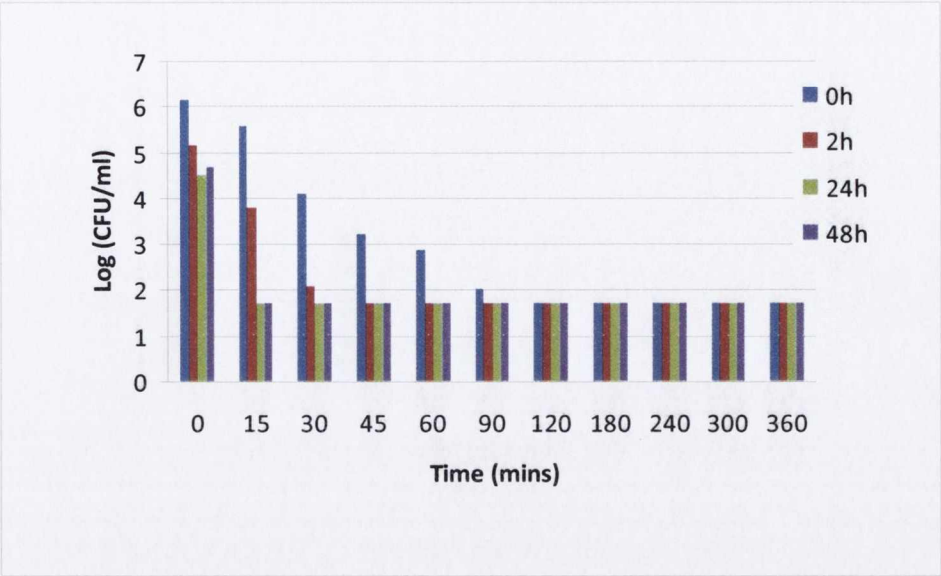
An investigation into post-irradiation activity was conducted for all configurations, as summarised in Table 5.1 and sample post-irradiation graphs can be seen in Figure 5.7. Control experiments were also carried out on a fully covered reactor. In these experiments, relatively little die-off occurred, despite the bacteria having no substrate on which to feed over the 48 h period.

When all six pipes of the reactor were fully open and no catalyst was employed, the bacterial die-off was more pronounced. For the sample taken at 120 minutes, the bacterial count reached the MDL after 48 h in the dark. No regrowth was witnessed, although the bacteria were not kept in ideal growth conditions, as they remained in distilled water and lacked the substrate and ions necessary for nutrition and osmotic balance. The EDT₂₄ was found to be 180 min, while the EDT₄₈ was found to be 120 min. However, for the photocatalytic test, the post-irradiation data shows that there was a much sharper decline in bacterial numbers during the time in the dark.

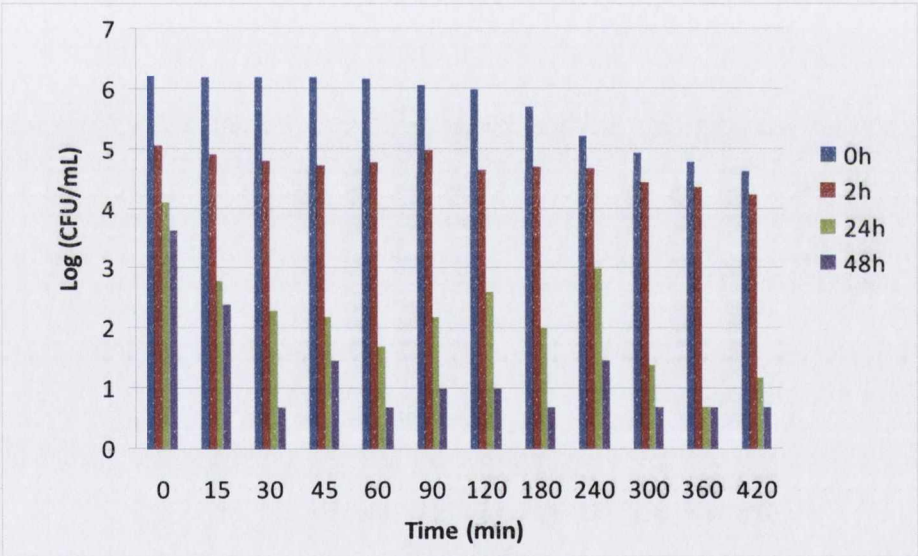
The Effective Disinfection Times calculated for experiments where the illuminated volume was kept the same (summarised in Table 5.2) reflect the findings for inactivation times in sections 5.3.1.2 and 5.3.2.2. In photolytic experiments the EDT₂₄ was either reached after a long period of time, or not reached at all, and there was no definitive correlation between flash frequency and EDT₂₄, although the two lowest values were obtained at the two lowest flash frequencies. In photocatalytic tests, the EDT₂₄ improved (decreased) with increased flash frequency, with the optimal value of 15 minutes in configurations 5, which had 18 openings.

EDT ₂₄ [min] without catalyst	Flash Frequency [flashes/min]	EDT ₂₄ [min] with catalyst
	2.8	60
180	8.4	
180	16.8	45
> 420	33.6	30
> 420	50.5	15
360	117.8	

Table 5.2 Effective Disinfection Times for experiments in which net illuminated volume was kept constant and number of periods of exposure to light was varied.



(a)



(b)

Figure 5.7 Graphs showing remaining bacterial numbers after 0, 2, 24 and 48 h in the dark. (a) configuration 1, 50 mg/L TiO₂; (b) configuration 11.

6. PHOTOLYTIC AND PHOTOCATALYTIC INACTIVATION OF PHAGES

6.1 Introduction

As described previously, *E. coli* has been used as a target organism in the vast majority of experiments looking into photocatalytic disinfection (Benabbou *et al.*, 2007, Dunlop *et al.*, 2001, Ireland *et al.*, 1993, Matsunaga & Okochi, 1995, Salih, 2002, Sun *et al.*, 2002, Sunada *et al.*, 2002, Wei *et al.*, 1994). As well as *E. coli*, the inactivation of other bacteria has been investigated using TiO_2 as a photocatalyst (Block *et al.*, 1997; Ibanez *et al.*, 2003; Saito *et al.*, 1992; Kühn *et al.*, 2003). Less research has been conducted on the photocatalytic inactivation of viruses, although *Poliovirus 1* (Watts *et al.*, 1995), *phage MS2* (Sjogren & Sierka, 1994), and *B. fragilis bacteriophage* (Laot *et al.*, 1999) are among those which have been tested. For example, *Poliovirus 1* inactivation rate kinetics were shown to follow Chick's Law and the virus was inactivated more quickly than *E. coli* under the same conditions (Watts *et al.*, 1995).

Viruses are unique in that they have no independent metabolic activities, rather they must infect living host organisms in order to reproduce. A virus consists of RNA or DNA (and not both), contained in a protective protein coat. The binding site for attaching a virus to a host is located on the protein coat of the virus. Viruses attach themselves to host cells and inject their genetic materials into the cells' cytoplasm (Beckett, 1986). They use the host cell's mechanisms to reproduce themselves and the new viruses move on to infect other cells. If the binding site of a virus is attacked by a disinfection method, it can no longer recognise receptor sites on host cells.

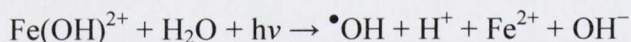
Early work on UV treatment of viruses concluded that DNA is the primary target for UV, while it was theoretically possible that the loss of unique genetic information is the main cause of inactivation. However, experiments suggested that the prevention of intracellular development preceding phage-coded expressions is the actual biological mechanism accounting for most of the inactivation (Lytle & Ginoza, 1969).

While *E. coli* is comprised of many complex systems, the inhibition of one of which can lead to deactivation, the simple, rigid structure of viruses makes them more difficult to disinfect in general (Cho *et al.*, 2005).

6.2 Bacteriophages studied

6.2.1 MS2

Phage MS2 is a single-stranded, icosahedral RNA bacteriophage, which, like Φ X174, has overlapping genes. It appears to be the most widely studied bacteriophage in the field of UV and solar disinfection. MS2 was found to be highly resistant to UV treatment (filtered UV, of wavelength >295 nm) in a study conducted by Mamane *et al.* (2007), with no disinfection at a wavelength greater than 295 nm. When H_2O_2 was introduced, however, MS2 was found to be more sensitive to treatment than T4 and T7 phages. The authors concluded that, in general, disinfection due to $\bullet OH$ radicals is small compared to that due to UV irradiation, although for viruses some oxidative enhancements can improve disinfection efficiency. On the other hand, Sjogren and Sierka (1994) proposed that hydroxyl radical oxidation is the primary cause of the inactivation of MS2. They also showed that the process can be enhanced by the addition of ferrous sulphate, which provides the iron required for the Fenton reaction to proceed. The Fenton reaction produces additional hydroxyl radicals according to the reaction:



Koizumi & Taya conducted a study investigating the disinfection of phage MS2 in a bubble column reactor in the presence of TiO_2 . While no disinfection took place at a light intensity of 22 W/m^2 in the absence of catalyst, they found that disinfection occurred in the presence of TiO_2 and proceeded linearly with respect to irradiation time. They also varied the concentration of TiO_2 and discovered a near-linear relation between catalyst concentration and disinfection rate constant up to a saturation value (Koizumi & Taya, 2002).

Using the same apparatus, Sato & Taya (2006) investigated the effect of different crystalline structures of TiO_2 on the disinfection of MS2. They found that an anatase:rutile ratio of 70 % (by wt.) was the most efficient, with the reasons for this explained in Section 2.6.1 (Sato & Taya, 2006).

In order to investigate the role of hydroxyl radicals in the photocatalytic disinfection of MS2 and *E. coli*, Cho *et al.* (2005) performed experiments under UV light (300-420 nm) in which

hydroxyl radical scavengers, *tert*-butanol and methanol were added to illuminated TiO₂ suspensions containing the microorganisms. In the absence of the scavengers, MS2 was shown to be far more resistant to photocatalytic disinfection than *E. coli*, with 0.95 and 2.25 log inactivations, respectively, after 120 minutes of illumination. When the scavengers were introduced, no inactivation of MS2 was seen at all, although some inactivation of *E. coli* still occurred, probably due to the effect of direct UV, other reactive oxygen species such as superoxide and hydrogen peroxide, and surface hydroxyl radicals that might survive the effects of the scavengers. The fact that no disinfection of MS2 occurred despite the existence of some radicals on the surface of TiO₂ particles led the authors to suspect that MS2 is inactivated by only the bulk phase hydroxyl radicals. The adsorption of MS2 onto TiO₂ particles is inhibited by electrostatic repulsion between the two. The influence of hydroxyl radicals was further supported when the authors performed further tests in which Fe²⁺ ions were introduced. These induce the Fenton reaction and lead to the formation of further hydroxyl radicals, as discussed previously. In these tests, the inactivation rate of MS2 increased substantially in the absence of scavengers and there was still no disinfection in the presence of scavengers. The killing of MS2 is induced by the denaturing of the capsid protein by hydroxyl radicals. Again in this study, the disinfection of all plaque-producing viruses investigated was shown to follow first-order kinetics. The photocatalytic disinfection of PRD1, MS2 and ΦX174 was investigated more recently by Gerrity *et al.* (2009), who performed experiments at bench-scale and pilot-scale. They found that at bench-scale, disinfection was inhibited by the presence of catalyst and attributed this to the attenuation of light by the catalyst and the lack of adsorption of MS2 to TiO₂ particles. When the experiments were repeated at pilot-scale, the introduction of catalyst had very little influence on the disinfection rate, but the authors pointed out that the presence of catalyst is preferable in treatment as chemical pollutants will be destroyed simultaneously. The authors also looked into the influence of the capsid protein components on photocatalytic disinfection and found that phages with higher levels of alanine, glycine and proline seemed to be more susceptible to treatment.

6.2.2 ΦX174

Phage ΦX174 is an icosahedral single-stranded DNA bacteriophage, which infects *E. coli*. Its circular, single-stranded DNA molecule was the first genome ever to be sequenced (Hurst *et*

al., 2007). It was also the first example of a genetic element with overlapping genes. These provide efficient coding for all of its proteins and overlap due to the small size of the genome (Madigan & Martinko, 2006). In a study investigating the sensitivity of microorganisms to different wavelengths of light, Φ X174 had no shoulder region in the dose response curves, which, as the author suggested, is to be expected from a single stranded virus (Giese & Darby, 2000). Sommer *et al.* (2001) discovered that Φ X174 was more susceptible to UV (253.7 nm) treatment than B40-8, which in turn was inactivated more readily than MS2. They suggested that the type of nucleic acid, RNA, in MS2 could be the reason for its high resistance, as the uracil in RNA may be less photoreactive than the thymine in DNA. However, other similar RNA viruses had been found to be inactivated relatively easily by UV, while there were some DNA viruses which were quite resistant. They therefore suggested that it was a combination of factors including size and type of virion as well as type of nucleic acid that dictated the susceptibility to treatment. The authors also concluded that when investigating the inactivation of viruses, a variety of strains should be used as indicators. This, they explained, was due to the fact that thus far it was not possible to predict the sensitivity of viruses to UV treatment from their size, structure and type of nucleic acid. They recommended that Φ X174 was too susceptible to UV treatment to be used as an indicator virus, but that MS2, as the most UV resistant bacteriophage in their study, may prove effective as a conservative indicator of UV virucidal activity. The inactivation kinetics of MS2 and Φ X174 were seen to follow a first order reaction, demonstrating a single hit process. It must be noted that this study was carried at UV-C wavelengths, as opposed to UV-A in the case of the results presented in this chapter.

6.2.3 PR772

PR772 belongs to the *Tectiviridae* family of bacteriophages and was first isolated in the 1970s from a wastewater treatment system in South Africa (Coetzee *et al.*, 1979, Coetzee & Bekker, 1979). They are icosahedral, double-stranded DNA bacteriophages, containing a lipid membrane beneath the shell. They infect Gram-negative bacteria carrying certain plasmids and are pilus-specific (Lute *et al.*, 2004, Goyal *et al.*, 1987). Among their hosts are *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* (Bamford & Mindich, 1982). Members of this family of phages are remarkably similar to each other, even though they have been isolated independently in extremely diverse geographical locations. This led Coetzee and Bekker to conclude that PR4 and PR772 were, in fact, the same phage (Coetzee & Bekker,

1979). Members of the *Tectiviridae* group are thought to be good indicators for adenoviruses, as they are similar in terms of genome replication, capsid architecture and vertex organisation (Lute *et al.*, 2004). Unlike some other members of the family, such as PRD1, which has *Salmonella typhimurium* as a host, PR772 is grown on non-pathogenic *Escherichia coli*, thus giving it a practical advantage for laboratory testing. To the author’s knowledge, no disinfection studies have been carried out on PR772, however a relatively similar somatic double-stranded bacteriophage, P22, was used in solar photolytic and photocatalytic studies by Davies *et al.* (2009). They obtained an S₉₀ value (Global Solar Exposure required to achieve a 90 % reduction in phage concentration) of 1.5 MJ/m². This was not improved by the introduction of fixed or suspended TiO₂ and was significantly reduced when the transmittance of UV-B wavelengths was attenuated. Another similar double-stranded DNA bacteriophage, PRD1 [The genome sequences of PRD1 and PR772 were found to be 97.2% identical (Lute *et al.*, 2004)], was shown to be 2-3 times less resistant to UV treatment than MS2 in an early study (Meng & Gerba, 1996).

Bacteriophage	MS2	ΦX174	PR772	PRD1
Size	26 nm	25 nm	53 nm	64 nm
Genome length	3,569 bp	5,386 bp	14,964 bp	14,927 nm
Genetic material	ssRNA	ssDNA	dsDNA	dsDNA
Topology	Linear	Circular	Linear	Linear
Infection	Male-specific	Somatic	Somatic	Somatic
Isoelectric point	3.5-3.9	6.6	4.0-4.2	3.0-4.2
Bacterial host	<i>Escherichia coli</i> 15597	<i>Escherichia coli</i> 13706	<i>Escherichia coli</i> BAA-769	<i>Salmonella typhimurium</i> LT2
UV Dose required for 4-log inactivation (mJ cm ⁻²)	62	10	N/A	30

Table 6.1 Characteristics of bacteriophages used, as well as PRD1 (Adapted from Gerrity *et al.*, 2008, Coetzee *et al.*, 1979, Brorson *et al.*, 2008, Bolton & Cotton, 2008)

6.3. Materials and Methods

6.3.1 Laboratory scale tests under UV lamp

The bench-scale CPC described in Section 3.1.4.1 was used and experiments were conducted as explained in Section 3.2.3.1. 1 mL of each phage was spiked into the reservoir simultaneously and allowed to mix fully before the first sample was taken. Subsequent samples were taken 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 1500 minutes after the first sample. 50 mg/L of TiO₂, prepared according to Section 3.1.1 was used in each photocatalytic experiment. The photolytic and photocatalytic experiments were both carried out three times to ensure accuracy of results. Plots were made of log₁₀(bacterial inactivation) vs cumulative dose and time, as explained in Section 3.2.4.

6.3.2 Full scale tests under natural sunlight

In the experiments under natural sunlight, the CPC as described in section 3.1.4.2 was used. It was tilted due South at 53°21' to the horizontal, which corresponds to the angle of latitude of the location at which the experiments were undertaken (Trinity College, Dublin, Ireland). The reactor was set up according to Section 3.2.3.2. The reactor was covered while the virus samples were spiked into the lower reservoir. TiO₂ was also added at this point in the photocatalytic experiments. After five minutes, when complete mixing of the viruses had occurred, the first 1 mL sample was taken.

Note: the solar experiments were repeated a number of times, as phage titration methods yield no results for some phages on occasion, usually due to the top agar and bacterial host overlay being too thin, making the visibility of plaques too poor for cell counts. Sufficient experiments were conducted such that there were two sets of results for each phage in both the absence and presence of TiO₂.

6.3.3 Viruses

The three viruses used in these experiments - MS2 (F-specific coliphage), ΦX174 (somatic coliphage) and PR 772 (*Tectiviridae* coliphage) - were supplied by ATCC in freeze-dried form.

After hydration in suitable broths, the viruses were stored at 4 °C. Viruses were propagated following the procedures set out in Section 3.2.2.

6.4. Results and Discussion

6.4.1 UV photolytic and photocatalytic inactivation

PR 772 was found to be extremely resistant to both UV and UV photocatalytic treatment, with only minor decay witnessed in 25 hours of illumination [corresponding to 87.21 kJ/L (Figure 6.1)]. The inactivation rates in the absence and presence of catalyst were very similar, with the introduction of photocatalyst bringing about a slight decrease in inactivation rate. This would corroborate findings by Gerrity *et al.* (2008), who used the similar phage PRD1, as reported in Section 6.2.1. They attributed the poor performance of the photocatalyst to the limited adsorption of the viruses onto catalyst particles and the attenuation of light by the TiO₂. ΦX174 was deactivated much more readily, with the introduction of catalyst improving the disinfection rate. MS2 was found in this research to be the most susceptible to both photolytic and photocatalytic treatment and again was found to disinfect more readily in the presence of catalyst. This is in contrast to previous findings by Sommer *et al.* (2001), who found MS2 far more resistant to UV treatment than ΦX174. In that study, however, germicidal light (~253 nm) was used, as opposed to UVA, meaning that the influence of reactive oxygen species would be muted in relation to the direct DNA disruption. They also suggested that ΦX174 may be a poor indicator, due to its high susceptibility to UV treatment, a claim which these findings would not support. In the tests conducted during this study, the phages were all present simultaneously, meaning that they were all in competition for any reactive oxygen species produced. Thus, results may have differed if the experiments were carried out on each phage sample individually. The plots for each of the trials showed good linearity, with the exception of the MS2 plots. Linearity would be expected in plots based on tests on single stranded viruses under a constant UV source (Giese & Darby, 2000), but the MS2 plots in the absence and presence of a catalyst both show fast initial disinfection, followed by a tailing off period until the MDL is reached.

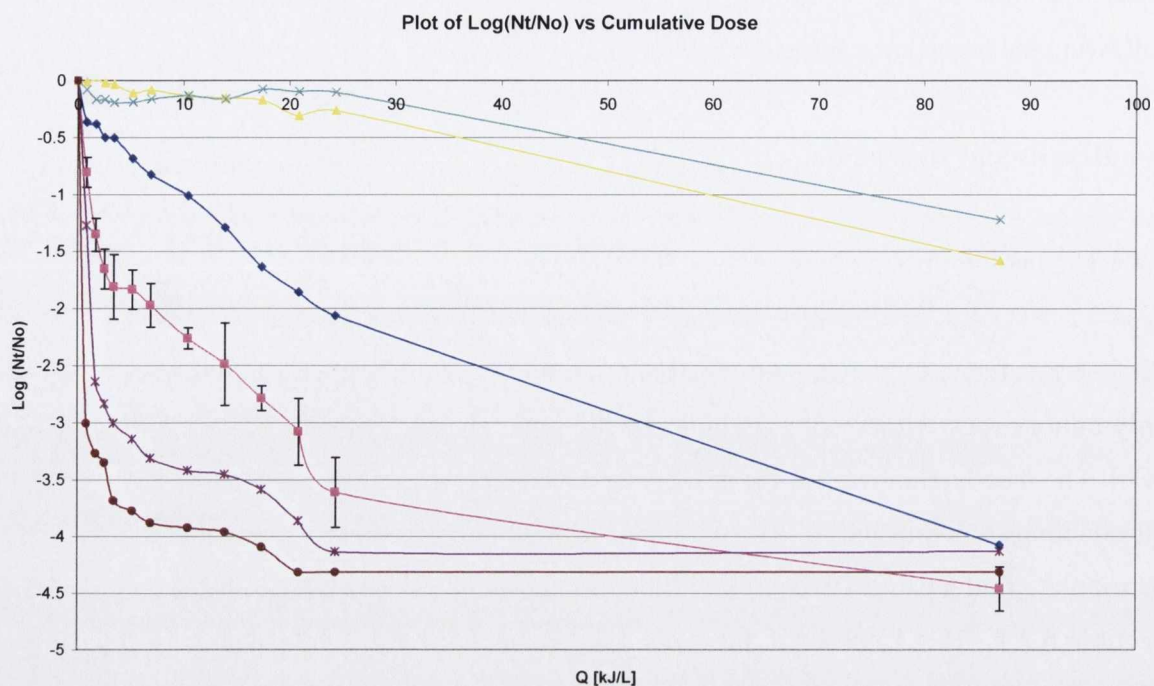


Figure 6.1 Plot of log disinfection vs cumulative UV dose for bench-scale experiments under UV light. ✕ MS2, 0 mg/L TiO₂; ● MS2, 50 mg/L TiO₂; ◆ ΦX174, 0 mg/L TiO₂; ■ ΦX174, 0 mg/L TiO₂; ▲ PR772, 0 mg/L TiO₂; × PR772, 50 mg/L TiO₂. (Note: error bars plotted on just one line to avoid confusion).

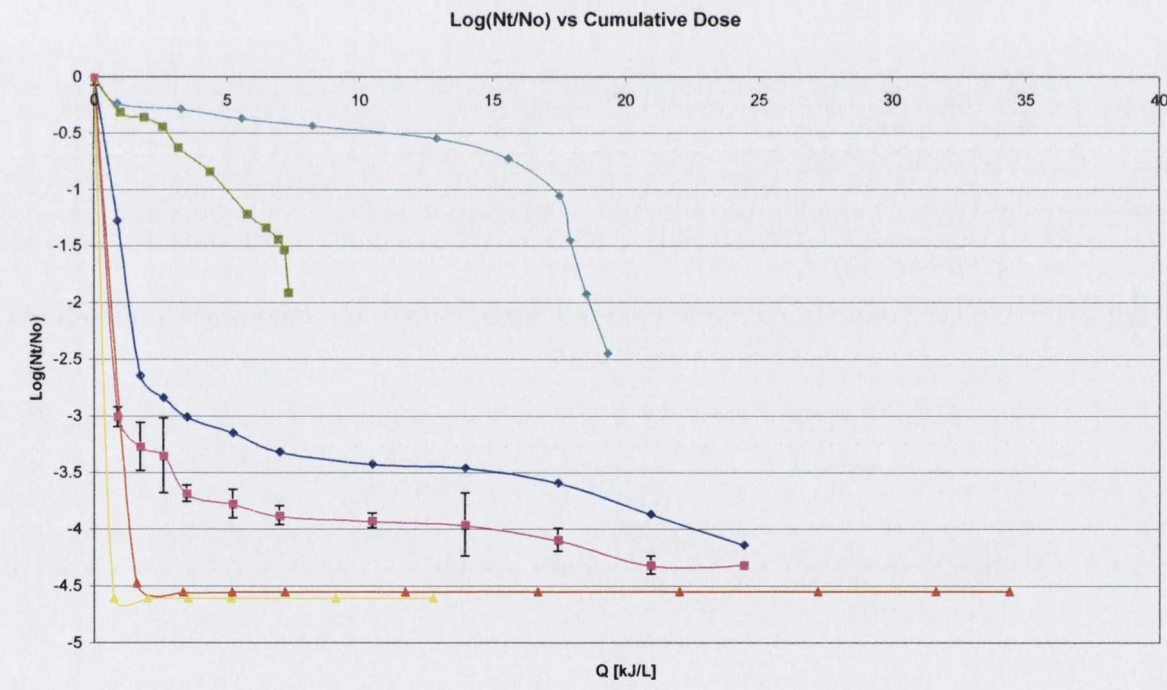
6.4.2 Solar photolytic and photocatalytic inactivation

The results of all experiments conducted under UV (in the bench-scale CPC) and sunlight (in the full-scale CPC) are compiled in Table 6.2 and compared in Figure 6.2. The time and cumulative UV dose to reach 1-log and 3-log inactivation in each case are presented. The days on which solar experiments took place ranged from completely overcast to very sunny.

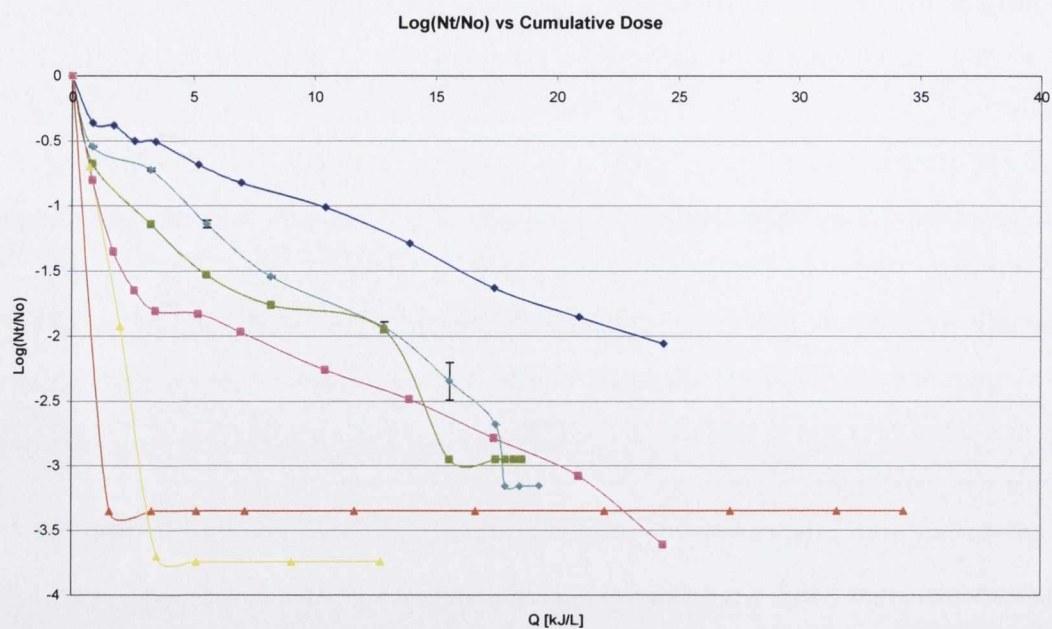
6.4.2.1 MS2

Under sunlight, the inactivation rate of MS2 was far slower than it had been under UV light, with respect to both time and UV dose received [Figure 6.2 (a)]. However, when photocatalyst was introduced, the initial inactivation rates were very similar to those seen in UV tests. Whereas there was a tailing off period during UV photocatalytic disinfection however, the

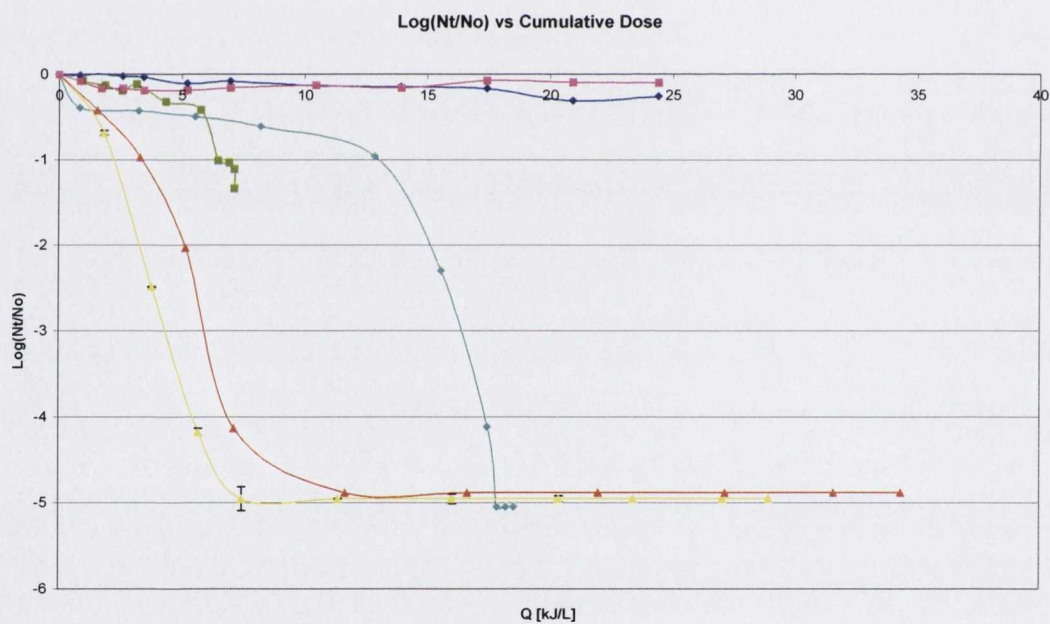
solar photocatalytic inactivation continued at the same rate until the MDL was reached. This linearity is more in keeping with previous research into the disinfection of phage MS2 by photocatalysis (Sjogren & Sierka, 1994). The higher disinfection rate in the presence of catalyst can be attributed to the far greater number of hydroxyl radicals produced during TiO₂ photocatalysis than during solar treatment in the absence of catalyst. As reported in Section 6.2.1, according to Cho *et al.* (2005) the photocatalytic disinfection of MS2 takes place solely due to the influence of bulk phase hydroxyl radicals, which induce the denaturing of the capsid protein. Sjogren and Sierka (1994) deduced that adsorption of MS2 to TiO₂ particles is not favoured at neutral pH, due to surface charges. Even the MS2 particles that would be forced into contact with TiO₂ particles by the water due to their hydrophobic nature would be situated at parts of the catalyst particle unsuitable for contact with hydroxyl radicals. Therefore, it would be expected that MS2 would be less readily inactivated if a fixed catalyst was used instead of suspended TiO₂.



(a)



(b)



(c)

Figure 6.2 Plots of bacterial inactivation vs cumulative dose comparing findings under artificial UV light and natural sunlight for (a) MS2; (b) Φ X174; and (c) PR772. $\blacklozenge$ 0 mg/L TiO_2 , UV; $\blacksquare$ 50 mg/L TiO_2 , UV; $\blacklozenge$ 0 mg/L TiO_2 , sunlight, first trial; $\blacksquare$ 0 mg/L TiO_2 , sunlight, second trial; $\blacktriangle$ 50 mg/L TiO_2 , sunlight, first trial; $\blacktriangle$ 50 mg/L TiO_2 , sunlight, second trial;

6.4.2.2 ΦX174

Disinfection of ΦX174 in the full-scale CPC under sunlight was more efficient than in the bench-scale CPC under UV light, both in the presence and absence of catalyst [Figure 6.2 (b)]. The solar intensity on all four days on which ΦX174 results were obtained was greater than the intensity received during the UV experiments, as is displayed in Table 6.1. It would appear that as well as cumulative dose, the intensity of light received is an important parameter for disinfection. That is, a short exposure to high intensity light is more efficient than a long exposure to lower intensity light, even if the cumulative dose is the same. It must also be remembered when comparing the UV and solar experiments that they were conducted in different reactor systems, with the full-scale system being a commercial production, optimized for optical efficiency and having a higher proportion of the circulating volume illuminated at any time. It is interesting that during both of the solar photolytic experiments the UV intensity decreased dramatically in the final two hours, but that disinfection proceeded rapidly. This might suggest that irreversible damage was done to some viruses during the high intensity period and that they subsequently gradually lost their viability.

Phage	TiO ₂ concentration [mg/L]	Light source	Time for 1-log inactivation [min]	Time for 3-log inactivation [min]	UV Dose for 1-log inactivation [kJ/L]	UV Dose for 3-log inactivation [kJ/L]	Peak UV intensity [W/m ²]	Average UV intensity [W/m ²]
MS2	0	UV lamp	~10	~60	~0.6	~3.5	17	17
ΦX174	0	UV lamp	~180	~900	~10.4	~52.3	17	17
PR772	0	UV lamp	~1000	nr	~58.1	nr	17	17
MS2	50	UV lamp	~5	~15	~0.3	~0.9	17	17
ΦX174	50	UV lamp	~22	~345	~1.3	~20.1	17	17
PR772	50	UV lamp	~1300	nr	~75.6	nr	17	17
MS2	0	Natural sunlight	~170	nr	~17	nr	39.3	22.5
MS2	0	Natural sunlight	~105	nr	~5	nr	18.2	6.6
ΦX174	0	Natural sunlight	~40	~190	~5	~18	39.3	22.5
ΦX174	0	Natural sunlight	~23	~120	~2	~14.5	45.5	22.7
PR772	0	Natural sunlight	~92	~145	~13	~16.5	45.5	22.7
PR772	0	Natural sunlight	~179	nr	~6	Nr	18.2	6.6
MS2	50	Natural sunlight	<5	<5	<0.25	<0.25	44.4	19.3
MS2	50	Natural sunlight	<5	<10	<0.5	<1	41.8	33.4
ΦX174	50	Natural sunlight	~20	~30	~1	~2.6	41.8	33.4
ΦX174	50	Natural sunlight	<5	<10	<1	<1	40.4	19.3
PR772	50	Natural sunlight	~20	~32	~2	~4.5	46.9	26.8
PR772	50	Natural sunlight	~32	~52	~3	~5.4	41.8	33.4

Table 6.2 Time and UV Dose required to reach 1-log and 3-log inactivation in each of the experiments. (Note: values are interpolated and values under UV light are averages from a combination of three repeat experiments). nr = not reached.

6.4.2.3 PR772

PR772 exhibited the most pronounced differences between inactivation rates under solar and UV light. Under UV light, 3-log disinfection was not achieved with or without photocatalyst after 25 h, or 87.21 kJ/L, of irradiation. In the solar experiments in the presence of photocatalyst, this was reached after just 32 and 52 minutes, or 4.5 and 5.4 kJ/L, respectively (these figures were interpolated from the results). Indeed, 5-log disinfection was achieved after 60 and 100 min, or 7 and 13 kJ/L. In the first photocatalytic trial, the average UV dose received was roughly 1.5 times that received during the photolytic experiments, while in the second trial, the average dose was twice the photolytic value. This would seem insufficient to explain the vast discrepancy in disinfection rates encountered. It is hypothesized that the vastly superior disinfection rates are linked to the high peak solar intensities received (46.9 and 41.8 W/m²), as well as the optical efficiency and proportion of volume illuminated in the large-scale reactor and the fact that the solar spectrum is far wider than the spectrum emitted by the UV lamp. Another factor which could be investigated in further studies would be the influence of temperature, as peak values of 33° and 37° were measured in the solar tests, while the temperatures did not rise above 27° under artificial UV light. It is an encouraging finding for the efficiency of solar photocatalysis that a species which was so resistant to UV photocatalytic treatment was so readily inactivated under sunlight. It must also be remembered that these tests, although conducted on sunny days, were performed at a significantly more northerly latitude (53°21') than most previous solar photocatalytic tests.

6.4.2.3 Comparison of disinfection kinetics under sunlight

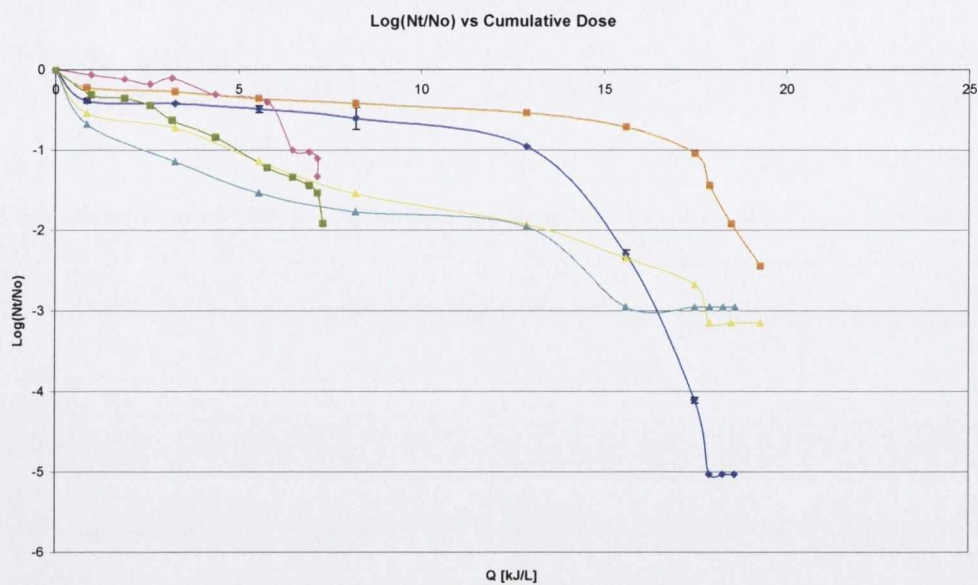
In Figure 6.3, the disinfection rates of the phages under solar treatment are compared. In the UV experiments, the viruses were seen to show the following relative resistance: PR772>ΦX174>MS2. Under natural sunlight, however, the inactivation rates were much more similar for each phage. Indeed, as Figure 6.3 (a) shows, it is difficult to draw a comparison among the phages in terms of efficiency of disinfection in the absence of catalyst. If the UV dose required for 1-log disinfection in each case is compared (Table 6.2), resistance declines in the following order: MS2 (Trial 1)>PR772 (Trial 1)>PR772 (Trial 2)>MS2 (Trial 2)≈ΦX174 (Trial 1)>ΦX174 (Trial 2). These findings would suggest that ΦX174 is the most susceptible to solar treatment, but further investigation shows that both ΦX174 trials and the

first PR772 trial were the only ones in which 3-log disinfection was reached. It is thought that the variation in intensity during the experiments had a major influence on the disinfection rate as the experiments progressed. In Figure 6.3 (b), it can be seen that the introduction of a catalyst brought about much more defined, linear inactivation kinetics. Again, this could be attributed to the production of hydroxyl radicals being less dependent on solar intensity than direct solar disinfection is. Again, as was found under artificial UV light, the MS2 phage was the most easily inactivated by photocatalysis, with PR772 the most resistant.

6.4.3 Comparison between disinfection of phages and *E. coli* under sunlight

A previous study investigated the differences between the photocatalytic treatment of MS2 and *E. coli* (Cho *et al.*, 2005). If the values obtained to reach 3-log disinfection of *E. coli* in the same reactor set-up in Chapter 4 are compared, it is apparent that *E. coli* was less resistant to solar treatment (2.5 kJ/L and 5.2 kJ/L required to achieve 3-log disinfection in two similar trials) than all of the viruses and more resistant to solar photocatalytic (3 kJ/L and 4.9 kJ/L) to all of the viruses, with the exception of PR772, with which it shared similar 3-log inactivation dose values. It would be expected that the more complex, less rigid structure of *E. coli* would render the bacteria less resistant to solar and solar photocatalytic treatment, as was discovered in the aforementioned paper. However, as the bacterial and viral experiments were not conducted on the same day, factors such as peak UV intensity, cloud cover and temperature must all be taken into consideration. Also, the catalyst concentration of 50 mg/L may not be idealized for disinfection of *E. coli* in the full-scale CPC.

The large variation in the disinfection characteristics of the different organisms under different experimental conditions suggests that a wide range of organisms should be used as indicators in similar studies. It also highlights the dangers of deeming particular phages as unsuitable indicator organisms on the basis of single experiments. For example, based on the solar experiments, PR772 may have been dismissed as too susceptible to photolytic and photocatalytic treatment to be of use as an indicator, yet it was by far the most resistant phage in the experiments conducted under artificial UV light. The findings in this chapter that MS2 was the most easily inactivated goes against the consensus seen in the literature (Section 6.2.1), which again shows that phages may behave very differently when experimental conditions are not exactly the same.



(a)



(b)

Figure 6.3 Plots of $\log_{10}(\text{disinfection})$ vs cumulative dose for experiments conducted under natural sunlight in the (a) absence and (b) presence of 50 mg/L TiO_2 . ■ MS2, first trial; ■ MS2, second trial; ▲ ΦX174 , first trial; ▲ ΦX174 , second trial; ◆ PR772, first trial; ◆ PR772 second trial.

7. THE MODELLING OF A SUITABLE FIXED PHOTOCATALYTIC INSERT GEOMETRY FOR USE IN A CPC

7.1 Introduction

The photocatalytic experiments undertaken in Chapters 3, 4, 5 and 6 were all conducted using titanium dioxide in suspension. Despite its superior efficiency to fixed catalysts, suspended TiO_2 requires costly and time-consuming filtration post-treatment and is an impractical option for implementing in water treatment systems. Thus, if an efficient fixed photocatalytic insert could be developed, it would greatly enhance the applicability of solar photocatalysis to water treatment in developing countries.

Compound Parabolic Collectors (CPCs) are ideally suited to solar photocatalysis and have been used in a number of disinfection studies (McLoughlin *et al.*, 2004, Fernandez *et al.*, 2005, Rincón & Pulgarin, 2007b, Gomes *et al.*, 2009) because they have turbulent flow regimes, high optical and quantum efficiency and can make use of both direct and diffuse radiation (Malato *et al.*, 2004). CPCs designed with a half acceptance angle (θ_a) of 90° can collect all direct and diffuse radiation reaching the collector (Malato *et al.*, 2004). In this case, the concentration factor of the CPC is 1 (non-imaging system), as

$$C = \frac{1}{\sin \theta_a} \quad (\text{Eqn. 7.1})$$

and the entire absorber area of the reactor is homogeneously illuminated, regardless of the weather conditions (Navntoft *et al.*, 2008).

As was alluded to in Section 2.7.5, far more literature is available on the computational modeling of UV reactors than on the modeling of solar systems. This is due to the widespread use of ultraviolet disinfection in large-scale water treatment facilities as well as the fact that the more predictable behaviour of UV light sources lends itself to more straightforward comparisons with experimental models.

In the research presented in this chapter, a variety of insert geometries were modeled in GAMBIT and examined using FLUENT Computational Fluid Dynamics (CFD) software. A section of the full-scale CPC reactor system (Section 3.1.4.2) was modeled, each insert was introduced and the flow of *E. coli* was simulated. Previous research was taken into account when sizing the geometries, as presented in Section 7.2.1. As photocatalytic disinfection relies on close contact between cells and TiO₂ particles (Guillard *et al.*, 2008), the percentage of particles coming into contact with the insert was determined for each geometry designed.

7.2 Methods

7.2.1 Geometry parameters

In a study into the optimization of solar photocatalytic collectors, Malato *et al.* (2004) presented the following equation, which takes into account the refractive index of the medium contained in the CPC:

$$C_{CPC,max} = \frac{na}{2\pi r} \quad (\text{Eqn. 7.2})$$

where $C_{CPC,max}$ is the maximum concentration factor of a CPC, r is the internal tube radius, n is the refractive index of the liquid passing through the tubes and a is the absorber area. The authors suggested that a practical application stemming from this equation would be the design of fixed photocatalytic inserts. In this case, the above equation could be used to calculate the smallest possible insert diameter whereby no optical losses would be experienced. This theory was put into practice in a subsequent paper (Fernández *et al.*, 2005), in which the authors stated that the optimum optical efficiency for a fixed photocatalytic insert is obtained when the ratio of the inner diameter of the CPC tube (D_i) to the external diameter of the concentric support (d_e) is equal to the refractive index of the fluid (n), or

$$n = \frac{D_i}{d_e} \quad (\text{Eqn. 7.3})$$

In the case of the full-scale reactor described in Section 3.1.4.2, the fluid has a refractive index of 1.33 and the internal diameter of the glass tubing is 46 mm. This gives an optimal external diameter of the insert of 34.58 mm. Although this optimizes the concentration ratio, it does not take into account particle residence time. As d_e increases, so does the cross-sectional area of the insert. This increases the water velocity around the insert and thus reduces the particle residence time in this region of flow. However, because the cross-sectional area of flow is reduced, particles will on average be closer to the insert than if the diameter was smaller.

As well as light refraction due to the medium, the light absorption effects of the medium can also be investigated using the Beer-Lambert formula. This formula for the light extinction in a well mixed layer of water states that:

$$I(z) = I_0 e^{-k_e z} \quad (\text{Eqn. 7.4})$$

where $I(z)$ is the intensity at a depth z from the water surface, I_0 is the intensity at the surface and k_e is an extinction coefficient.

Thus, it is thought that the ideal shape for the geometries would have:

- A large irradiated surface area, to optimise the number of available contact sites for *E. coli* and TiO_2
- A small cross sectional area, so that velocities are not too high (and thus bacterial residence times too low) in the vicinity of the insert
- A shape which promotes a degree of turbulence, to ensure *E. coli* are fully mixed
- A shape that lends itself to easy production for large scale processes

The material for the support needs to be relatively low density, non-reactive and watertight. Previously, materials used have included TiO_2 coated glassfibre paper wrapped around polypropylene supports (Fernández *et al.*, 2005).

7.2.2 Geometries tested

No insert (Model 1)

Primarily, a simulation was run with a 1 m long section of the glass tube set as the irradiated area under investigation. This simulates the effect of coating the interior of a tube with a transparent sol-gel catalyst in lieu of introducing an insert.

Cylinders (Models 2-5)

The first insert geometry tested was the solid cylinder conventionally used in photocatalytic reactors (Sichel *et al.*, 2007, Fernández *et al.*, 2005), with a radius of 17.29 mm, as derived from Equation 7.3. Three other sizes of cylinder were considered, with radii of 10 mm, 15 mm and 20 mm, to investigate whether the idealised optical design would also yield the most favourable disinfection characteristics in comparison to other sizes.

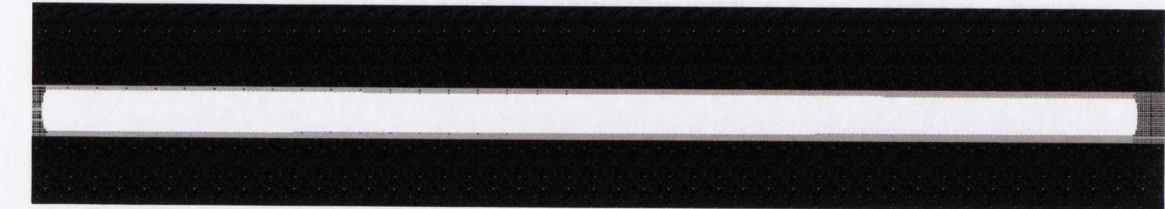
Conical Frusta (Models 6-9)

The solid cylinder has a large irradiated surface area, but because the surface lies in a plane parallel to the flow of water, the number of bacteria coming into contact with it is not optimised. To this end, two conical frustum shapes were conceived as being possible alternatives to cylinders. The radius in the middle of each frustum was kept at 17.29 mm. The first frustum had a minor radius of 16.29 mm and a major radius of 18.29 mm, while the second had a minor radius of 14.29 mm and a major radius of 20.29 mm. Each frustum was modeled twice, once with the radius increasing in the direction of flow (models 6 & 8) and once with the radius decreasing in the direction of flow (models 7 & 9). As an example, the shape of model 7 is displayed in Figure 7.1 (a).

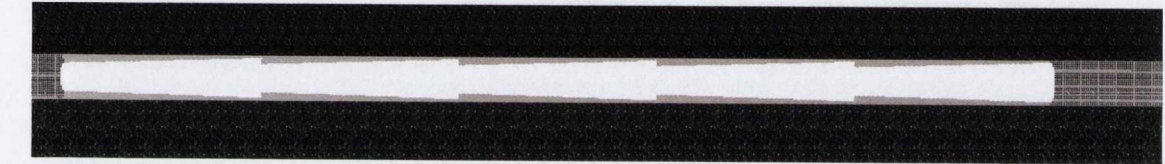
Consecutive frusta (Models 10-13)

In order to introduce a greater level of turbulence by further interrupting the cross-sectional area of flow, geometries were created in which 5 frusta were placed end-to-end. This was

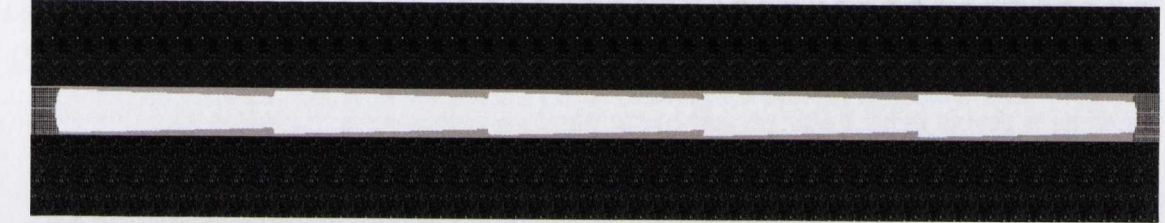
conducted using the same radii as in models 6-9 and examples of such shapes can be seen in Figures 7.1 (b) & (c).



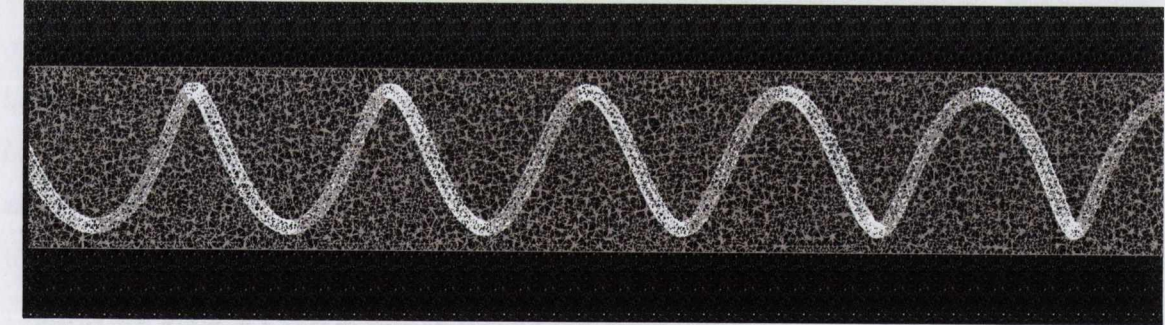
(a)



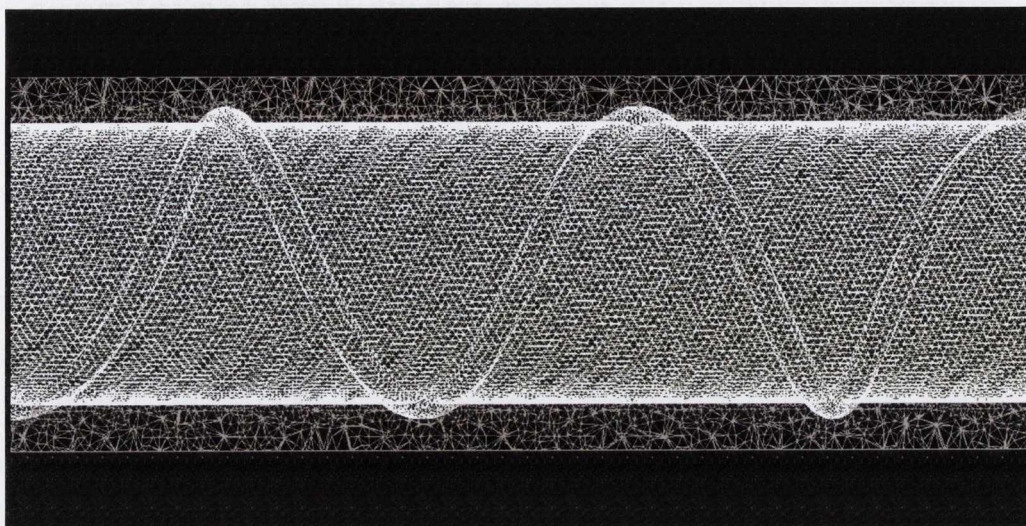
(b)



(c)



(d)



(e)

Figure 7.1 Examples of insert geometries (a) Frustum (model 7); (b) arrangement of 5 frusta (model 10); (c) arrangement of 5 frusta (model 13); (d) spring (model 14); (e) cylinder merged with spring (model 15). Note: images are magnified and a plane through the reactor along the $z = 0$ plane is drawn in each case to provide scaling.

Spring (Models 14 & 15)

Due to the relatively high cross sectional area associated with a conventional cylindrical insert, the flow surrounding the insert is constricted significantly, increasing the velocity and reducing the residence time in the region of the insert. Therefore, a spring geometry was also created, which had a shape conducive to the creation of turbulence as well as a low cross sectional area in the direction of flow. A tube of radius 2 mm was constructed along a helix of radius 17.29 mm. Finally, the aforementioned helical shape was wrapped around the cylinder with radius 17.29 mm, to investigate whether the added turbulence increased the number of *E. coli* ‘particles’ coming into contact with the insert.

7.2.3 Creation of geometries in GAMBIT

The full-scale CPC (Section 3.1.4.2) was measured in detail and a three-dimensional technical drawing was created in GAMBIT (Figure 7.1). For the purposes of modelling, to vastly reduce the amount of CPU memory and time required to run simulations, a two-pipe section of the

reactor was utilised. This section included a detailed simulation of a complex bend from the CPC, which is shown in Figure 7.3, to ensure realistic turbulence profiles would be created.

A variety of geometries, as detailed in Section 7.2.2, were then designed and constructed using GAMBIT. Each geometry was designed with a length of 1 m, to facilitate comparisons among the geometries. The geometries were constructed and meshed using GAMBIT, with a mesh size of 1 mm in the region of the insert and 2 mm in the rest of the reactor model. This enabled the accurate calculation of flow profiles and particle trajectories once the geometry was exported to FLUENT.

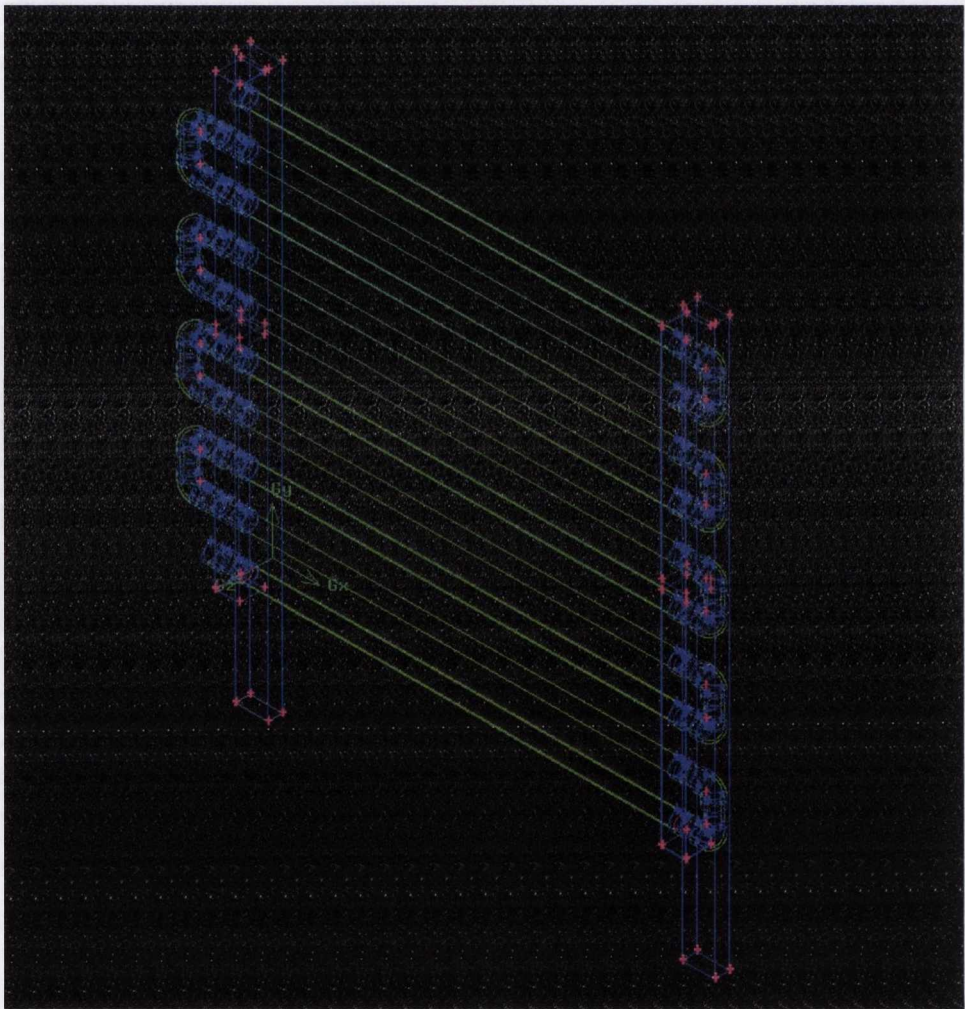


Figure 7.2 Technical drawing of the full-scale CPC in GAMBIT

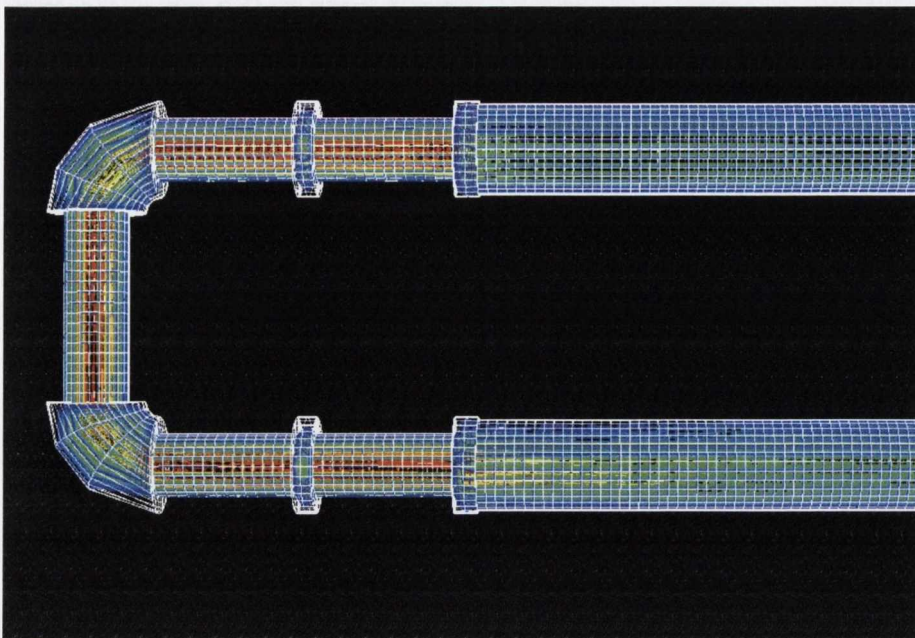


Figure 7.3 Complex bend connecting pipes

7.2.4 Simulation of flow in FLUENT

FLUENT 6.3.26 was the Computational Fluid Dynamics (CFD) software used in the modelling of flow in the reactor system. The standard $k-\epsilon$ turbulence model was used in all simulations. Each mesh was read into FLUENT and after the grid had been checked and scaled, grid interfaces were defined and water-liquid was selected from the FLUENT database of materials. Operating and boundary conditions were then inputted as follows:

Operating Conditions

Gravity = $-9.814 \text{ m}^2/\text{s}$ (y-direction)

Pressure = 101,325 Pa (atmospheric)

Boundary conditions

Velocity inlet: The inflow was chosen to be the top right of the two-pipe model (see Appendix B for diagrams), as water flows from top to bottom in the full-scale CPC (Section 3.2.3.2),

meaning that the inlet velocity was inputted as a negative number. The velocity was calculated by dividing the flow rate by the internal cross-sectional area of the tube.

$$x\text{-velocity} = -0.0933 \text{ m/s}$$

The turbulent intensity was calculated from Eqn 7.4.

$$I \equiv \frac{u}{u_{ave}} = 0.16(\text{Re}_{D_H})^{-1/8} \quad (\text{Eqn. 7.5})$$

(FLUENT, 2006)

where Re_{D_H} is the Reynolds number of the flow regime.

$$\text{Re}_{D_H} = \frac{\rho \times v \times D_H}{\mu} \quad (\text{Eqn. 7.6})$$

where ρ = fluid density (1000 kg/m³); v = velocity of flow = 0.093 m/s; D_H = hydraulic diameter (see Equation 7.7) and μ = fluid viscosity = 8.9×10^{-4} Pa s for water at 25 °C.

The hydraulic diameter is calculated from the following equation:

$$D_H = \frac{4A}{p} \quad (\text{Eqn. 7.7})$$

where A is the internal cross sectional area of the pipe and p is the wetted perimeter. In this case, as for all circular cross-sections, the hydraulic diameter is equal to the internal diameter, as p is equal to the internal circumference, Thus,

$$D_H = \frac{4A}{p} = \frac{4 \times \pi r^2}{2\pi r} = 2r = D \quad (\text{Eqn. 7.8})$$

Hydraulic diameter = 0.046 m, which, when substituted into Eqn. 7.6, gives a Reynolds number of 4821, indicating turbulent flow. The turbulent intensity, calculated from Eqn. 7.5, is, therefore, 5.54 %, again a value associated with turbulent flow.

Outflow: The outflow was set as a pressure-outlet and was situated at the bottom right of the two-pipe model. Again the turbulent intensity and hydraulic diameter were set at 5.54 % and 0.046 m, respectively.

Fluid: The fluid was set as the previously selected water-liquid.

The model was then initialized and iterated until convergence. The relative residuals were selected as 1×10^{-4} to ensure good convergence would be achieved. The velocity and turbulence intensity profiles were checked and contours of these parameters were displayed. Once the flow field was satisfactory, the discrete phase model was implemented.

7.2.5 Particle tracking in FLUENT

Discrete Phase Modelling (DPM)

For the discrete phase model, *E. coli* was set as a user-defined material, with density 1100 kg/m³ (Baldwin *et al.*, 1995), and diameter 1×10^{-6} (observed in Section 4.3.5).

A surface injection was chosen, whereby one particle was released from each mesh area on the inflow surface, giving 406 particles in total. The 'Interaction with continuous phase' setting was chosen. For the discrete phase model, the boundary conditions of the irradiated surface area were set to 'trap'. This meant that any particle coming into contact with the insert would be reported in the console window as 'trapped', thus allowing the quantification of *E. coli* interaction with the irradiated area of each geometry.

7.2.6 Limitations of CFD modelling

The CFD modelling regime undertaken provides an interesting comparison of the efficiency of supports of different shapes. It does, however, need to be followed up with a regime of

experiments on the inserts in order to investigate the influence of a number of factors not incorporated into the modelling, namely:

- catalyst sloughing
- the accumulation of biofilms on inserts and hence the reusability of inserts
- the most effective support materials
- cell/catalyst surface charge effects

7.3 Analysis of Models

Velocity profiles, turbulence profiles and sample single particle track pathlines are presented in Appendix B and results are summarised in Table 7.1.

7.3.1 No insert

The model run without an insert, with a 1 m section of pipe set as the irradiated area, exhibited the poorest performance in terms of particle hits, with none of the tracked particles coming into contact with the area under question. The high degree of turbulence created as the fluid moved around the bend dissipated relatively quickly and did not promote a high degree of mixing in the reactor tube.

7.3.2 Cylinders

For the cylindrical insert, the attenuation of the intensity of radiation reaching the insert surface can be calculated relatively simply from the Beer-Lambert Law (Eqn. 7.4):

$$I(z) = I_0 e^{-k_e(r_t - r_i)} \quad (\text{Eqn. 7.9})$$

where r_t is the internal radius of the reactor tube and r_i is the radius of the cylindrical insert. Note: I_0 here refers to the intensity of light at the internal surface of the reactor tube. Typical values of k_e presented in literature have ranged from 0.03 m^{-1} in pure water to 4 m^{-1} in highly stained lakes (Martin *et al.*, 1999). Even for the smallest insert radius (10 mm) at an extremely

conservative extinction coefficient of 0.5 m^{-1} , the intensity reaching the catalyst is only reduced by 0.65 %, meaning this effect is negligible due to the small diameter of the tubing.

The optically idealised cylinder (Model 2) trapped 8.9 % of particles flowing around it, which would seem to confirm that a fixed catalyst on such a cylinder would yield better photocatalytic disinfection than a coated tube. This value can also be used to compare the efficiencies obtained for different

The cylinder with radius 10 mm (Model 3) showed the worst performance in terms of particles coming into contact with the irradiated surface area (2.7 %), with the exception of the set-up in which no insert was included. While turbulence would be expected to be quite high due to the low velocity in the region of the insert, it was found that turbulence increased with insert size. This effect is due to the sudden constriction of the flow and the edge losses as the flow comes into the insert region. The irradiation received by the 10 mm radius cylindrical insert would also be lower than for the 17.29 mm, as the refractive index of water affects the concentration factor according to Eqn. 7.2.

When the radius was raised to 15 mm (Model 4), the number of particles striking the irradiated surface increased, which would be expected from the limited cross-sectional area through which the particles were able to flow. As mentioned above, the turbulence also increased due to the sudden constriction of flow.

Finally, when a radius of 20 mm was used (Model 5), imposing an average fluid velocity of 0.38 m/s around the insert, 13.4 % of particles came into contact with the hypothetical TiO_2 sites. but the residence time for the particles in the 1 m section was relatively low. From Chapter 4, it may be thought that the lower particle residence time in the vicinity of the insert does not necessarily imply a detrimental effect on photocatalytic disinfection rates, but may have an effect on photolysis. However, these previous studies were conducted using suspended TiO_2 . In a paper by Sichel *et al.* (2007), increased flow rate was seen to have a negative impact on disinfection rates with respect to UV dose in fixed photocatalysis, a phenomenon that was even more pronounced when disinfection in dark experiments was taken into account. The authors attributed the effect to better interaction between cells and catalyst particles at slower flow rates. Thus, the influence of the synergistic effect of flow rate and

Insert	Shape	Illuminated area (cm ²)	Insert volume (cm ³)	Average cell velocity along insert (m/s)	Average residence time in insert section (s)	% of particles trapped by insert	Major dimensions (mm)
1	No insert	1445.1	1661.9	0.093	10.72	0.0	(r = 23)
2	Cylinder	1086.4	939.2	0.214	4.66	8.9	r = 17.29
3	Cylinder	628.3	314.2	0.115	8.69	2.7	r = 10
4	Cylinder	942.5	706.9	0.162	6.16	8.7	r = 15
5	Cylinder	1256.7	1256.6	0.382	2.61	13.4	r = 20
6	Frustum	1086.4	940.2	0.215	4.66	10.5	r ₁ = 16.29, r ₂ = 18.29
7	Frustum	1086.4	940.2	0.215	4.66	10.1	r ₁ = 16.29, r ₂ = 18.29
8	Frustum	1086.9	948.6	0.217	4.60	31.0	r ₁ = 14.29, r ₂ = 20.29
9	Frustum	1086.9	948.6	0.217	4.60	17.1	r ₁ = 14.29, r ₂ = 20.29
10	5 x Frustum	1086.4	940.2	0.215	4.66	11.1	r ₁ = 16.29, r ₂ = 18.29
11	5 x Frustum	1086.4	940.2	0.215	4.66	11.9	r ₁ = 16.29, r ₂ = 18.29
12	5 x Frustum	1086.9	948.6	0.217	4.60	34.0	r ₁ = 14.29, r ₂ = 20.29
13	5 x Frustum	1086.9	948.6	0.217	4.60	17.4	r ₁ = 14.29, r ₂ = 20.29
14	Spiral	286.2	27.3	0.095	10.55	11.3	tube r = 2, helix r = 17.29
15	Cylinder within spiral	1092.3	953.5	0.220	4.57	9.8	tube r = 2, helix r = 17.29, cylinder r = 17.29

Table 7.1 Summary of insert geometries

number of particles contacting the irradiated area needs to be investigated in an experimental comparison of the inserts designed. As the light intensity and concentration factor would not show significant variation between the 20 mm and 17.29 mm inserts, it could be concluded that for photocatalytic applications, increasing the insert radius could improve photocatalytic disinfection rates.

7.3.3 Frusta

For the frusta, an integration must be carried out to determine the average intensity. The variation in cross-sectional area as well as the depth variation along the length of the frustum needs to be considered. In this case, the integration along the length of the frustum can be expressed as:

$$\bar{I} = \frac{\int_0^L I_0 e^{-k_e \left\{ r_1 - \left[r_1 + \frac{(r_2 - r_1)l}{L} \right] \right\}} x 2\pi \left(r_1 + \frac{(r_2 - r_1)l}{L} \right) \cdot dl}{A} \quad (\text{Eqn. 7.10})$$

where r_1 is the minor frustum radius, r_2 is the major frustum radius, L is the perpendicular length of the frustum and A is the frustum area, which can be obtained from:

$$A = \pi(r_1 + r_2)s \quad (\text{Eqn. 7.11})$$

Here, s is the slant height, easily obtained from Pythagoras's theorem:

$$s = \sqrt{h^2 + (r_1 - r_2)^2} \quad (\text{Eqn. 7.12})$$

Eqn. 7.10, upon integration, gives

$$\bar{I} = \frac{2\pi I_0 L e^{-k_e r_1 + k_e r_1}}{A k_e} \left[\left(\frac{r_1 L}{r_2 - r_1} + L - \frac{L}{k_e (r_2 - r_1)} \right) e^{\frac{k_e (r_2 - r_1)l}{L}} \right]_0^L \quad (\text{Eqn. 7.13})$$

Inputting the same k_e value of 0.5 m^{-1} again shows negligible light attenuation within the fluid, with less than 0.3 % attenuation in both frustum models.

The frustum with minor radius 16.29 mm and major radius 18.29 mm (Model 6) displayed better performance in terms of particle hits (10.5 %) than the cylinder with radius 17.29 (8.9 %), despite having almost identical volume and irradiated surface area. In the case of the frustum, as the cross-sectional area of flow available to the particles gradually decreases along the length of the frustum, they are more likely to come into contact with the irradiated area. When the direction of the frustum was reversed, in Model 7, the number of particles tracked was almost exactly the same. As can be seen in Figure 7.4, the turbulent intensity is very high as the flow is constricted when it comes into contact with the insert, promoting more mixing of the fluid and encouraging cells to come into contact with the insert. However, as the insert radius then decreases in the direction of flow, the cells will move in the x-direction and the shape of the insert is then not conducive to contact with the particles.

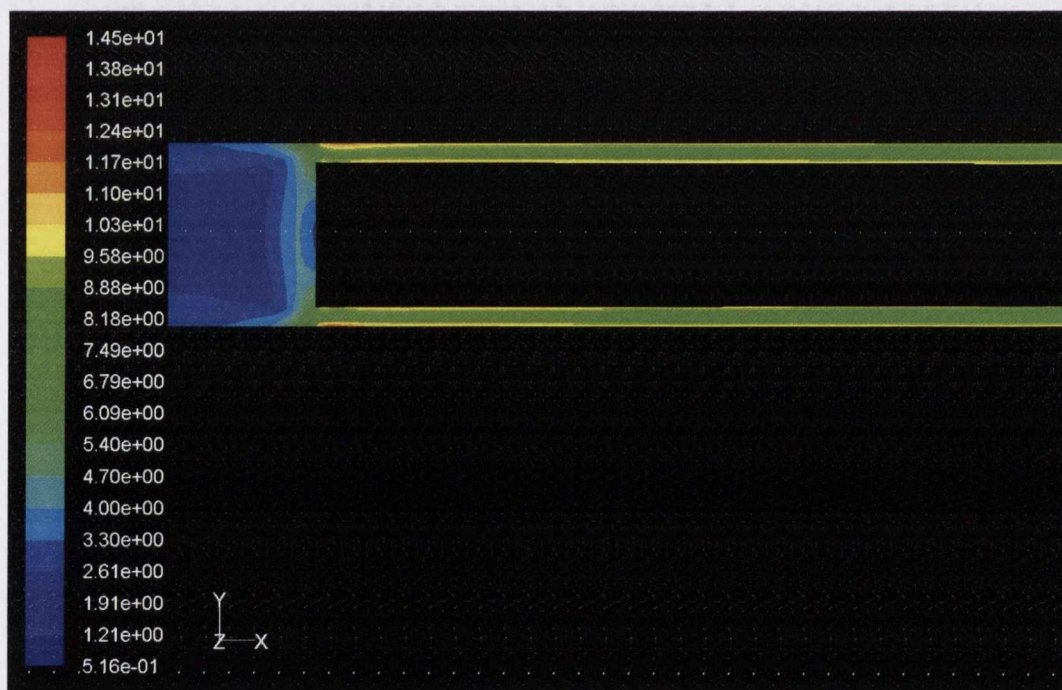


Figure 7.4 Contours of turbulent intensity (%) along central plane ($z = 0$) for Model 7. The image is a close-up of the section of the pipe where the insert (darkened area) starts.

When the dimensions of the frustum were changed to a minor radius of 14.29 mm and a major radius of 20.29 mm (Model 8), the performance in terms of number of particles trapped by the geometry increased dramatically (31 %). As can be seen in Table 7.1, the irradiated area and volume were only slightly larger than that of the previous frustum examined and the cylinder with radius 17.29 mm (and hence the residence time along the insert is only slightly smaller). This means that the much higher number of particles trapped is down to the difference in shape, rather than size, of the inserts. Any particles travelling in the x-direction will tend to be caught if they do not deviate, or only deviate slightly from flow in the x-direction. As the flow becomes more and more constricted along the length of the insert (Figure 7.5), turbulence will increase, promoting more contact between cells and the insert wall. This frustum shape could be developed as a possible alternative to the more conventional cylinders used as supports for fixed photocatalysts in CPC reactors.

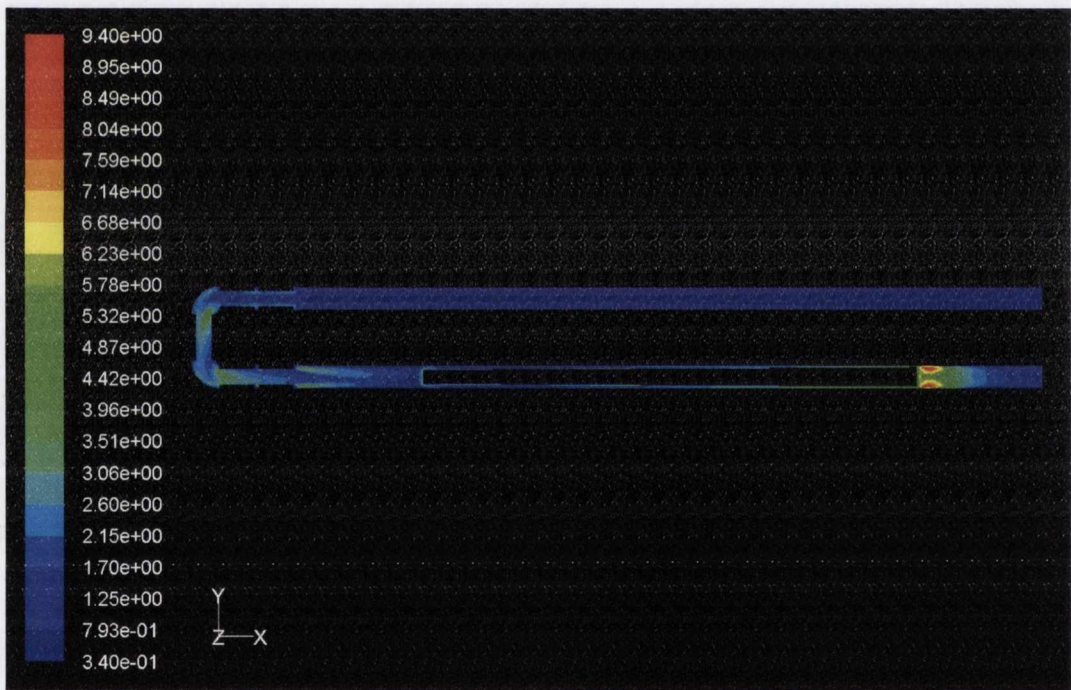


Figure 7.5 Contours of turbulent intensity (%) along central plane ($z = 0$) for Model 8.

When this frustum is reversed (Model 9), the high degree of turbulence caused by the significant sudden constriction of the flow (Figure 7.6) is negated by the tapering of the insert in the direction of flow, which is not conducive to contact with cells travelling in the positive x-direction.

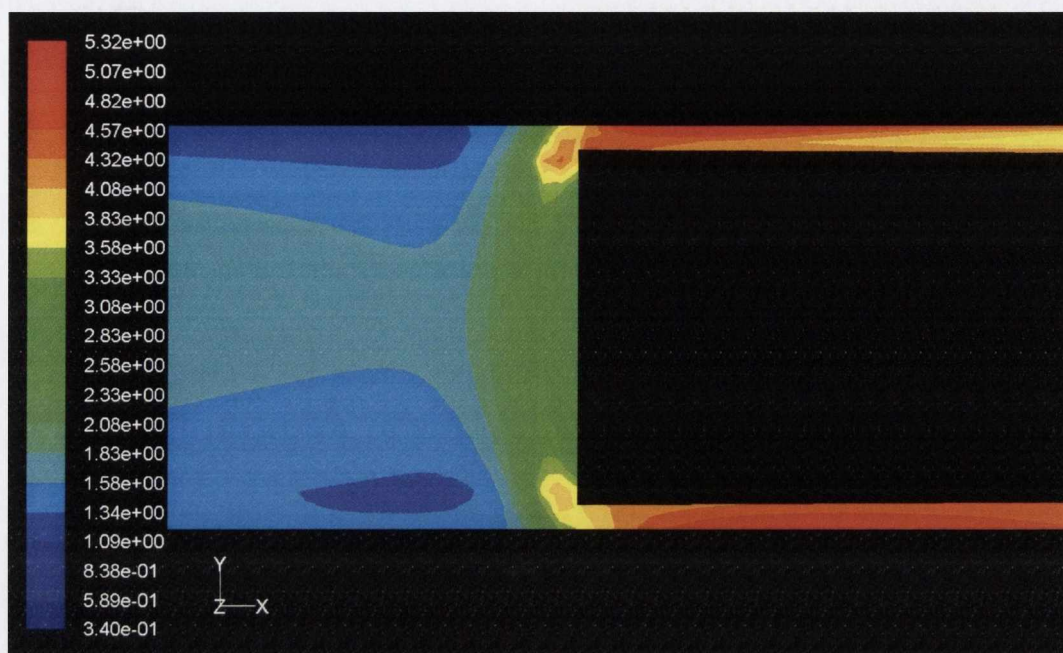
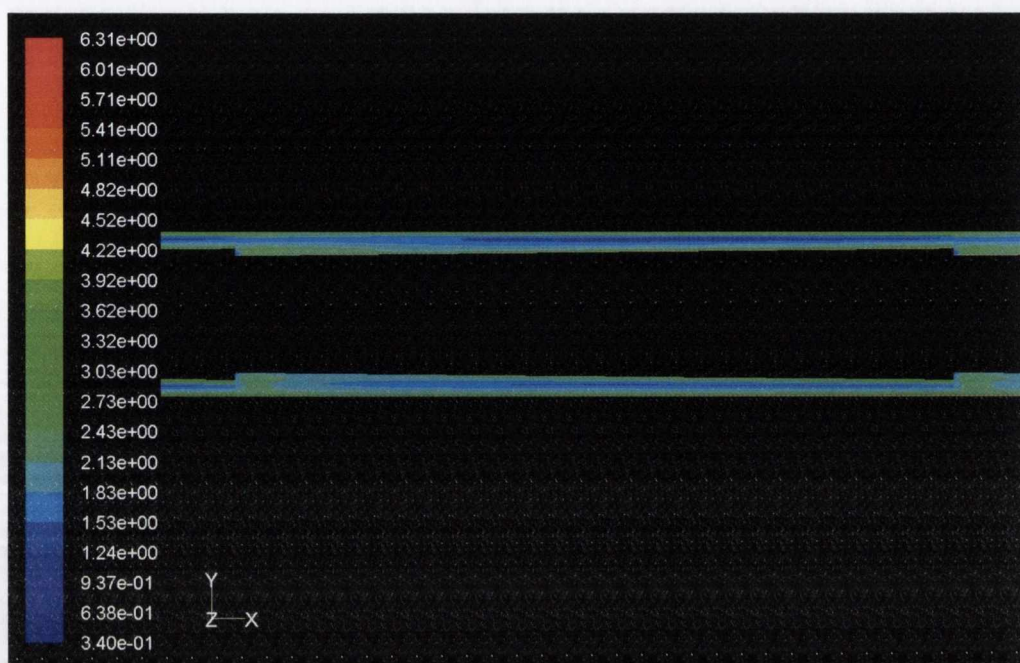


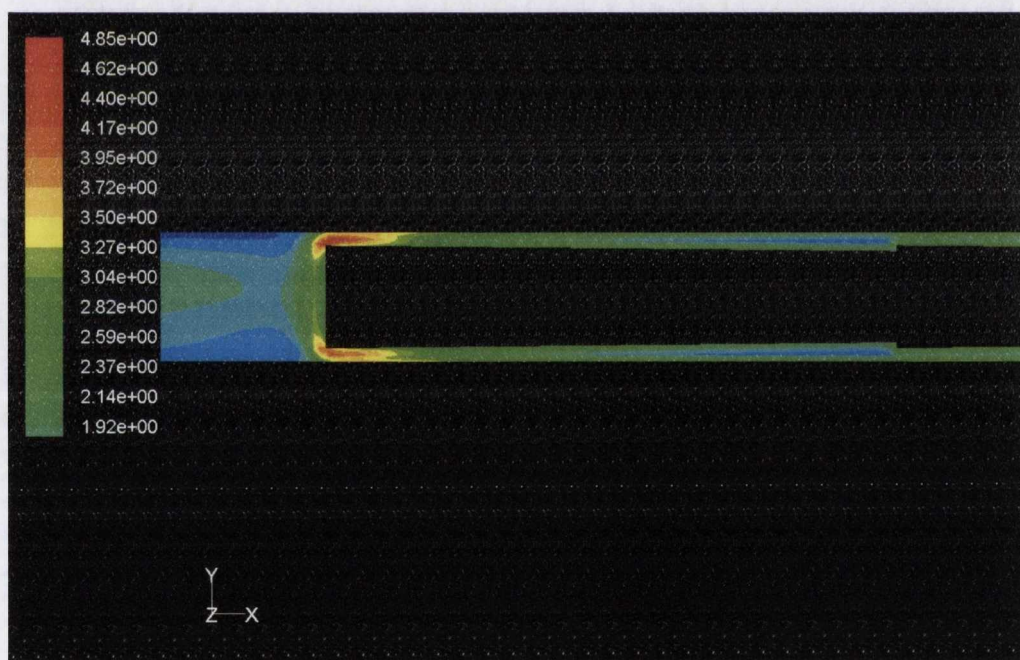
Figure 7.6 Increase in turbulence intensity (%) due to flow constriction at the start of the insert (Model 9)

7.3.4 Consecutive frusta

The consecutive frusta models were conceived in order to increase the number of areas of high turbulence along the length of the insert. It also increases the steepness of the slant height for the frusta, s (Eqn. 7.12), as the radius increases by the same degree as before over a shorter perpendicular length (200 mm, as opposed to 1,000 mm). The light attenuation was worked out using Eqn. 7.13, and again was seen to be negligible. Model 10 showed slightly improved particle hit results (11.1 %) when compared with the single frustum with the same radii (Model 6, 10.5 %). This can be attributed to the increased turbulence at each of the intersections of the five frusta, as well as the steeper profile discussed above. When this multi-frusta arrangement was turned around, so that the radius decreased in the direction of flow (Model 11), there was a slight increase in the number of particle hits (11.1 %). From Figure 7.7, it can be seen that the turbulence intensity at the intersections for Model 11 was slightly greater than that for Model 10. This would appear to outweigh the positive effect on particle hits that would be brought about by the increasing radius in Model 10.



(a)



(b)

Figure 7.7 Turbulence intensity (%) contours at intersections of frusta; (a) Model 10; (b) Model 11)

When the dimensions used in Models 8 and 9 were applied to the consecutive frusta (Models 12 & 13), the number of particle hits in both cases exceeded those for the respective single frustum profiles. Figure 7.8 shows that a very high turbulence intensity is created at the frustum intersections. Again, this leads to greater radial variation of particle trajectories and increases the likelihood of cells contacting the irradiated surface. Coupled with the effect of the steeper slope of the irradiated area, this explains why the highest number of particle hits (34 %) was achieved by this design. When the arrangement was reversed (Model 13), the turbulence intensity was extremely high again, but the steep tapering of the insert radius in the direction of flow explains the less effective performance (17.4 %) in relation to Model 12.

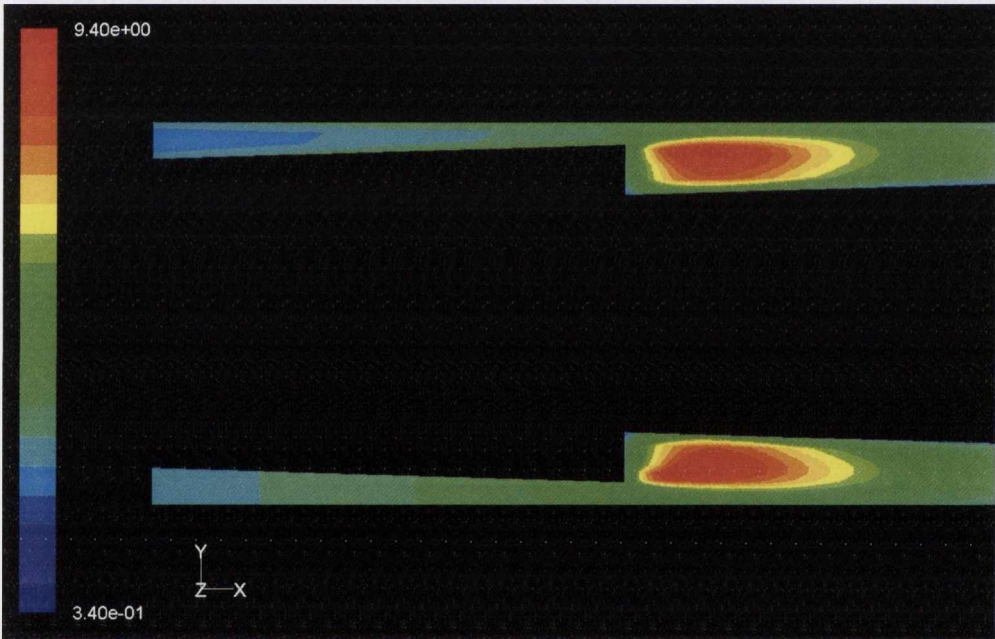
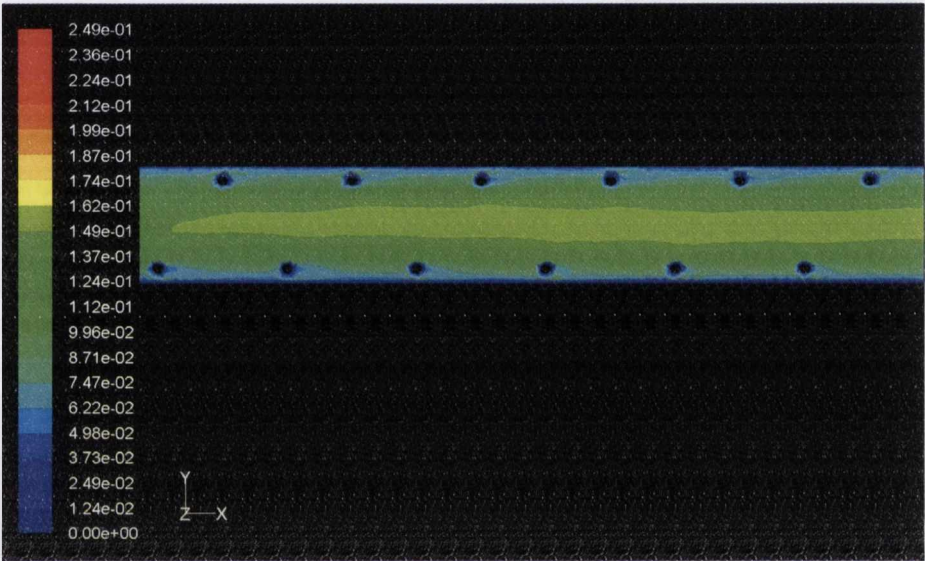


Figure 7.8 Turbulence intensity (%) contours for Model 12

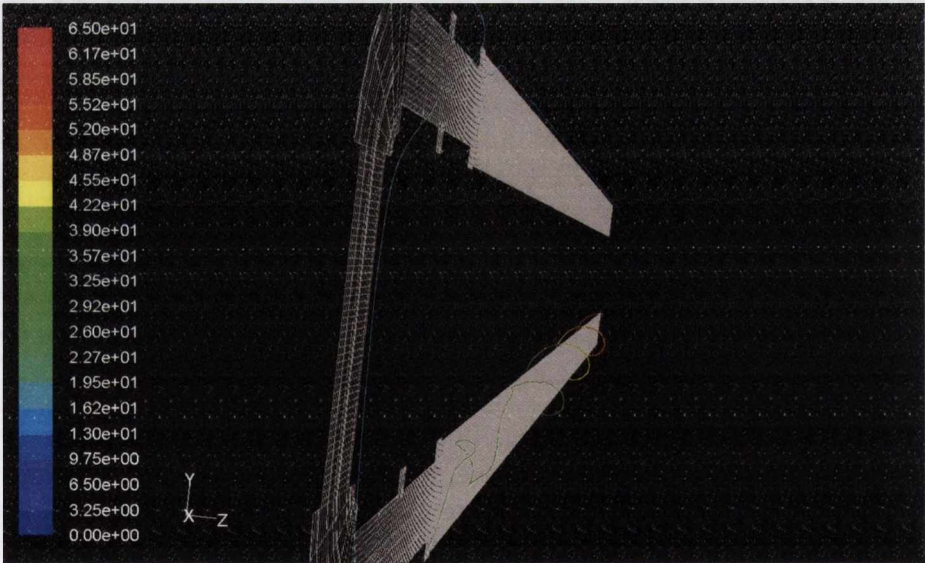
7.3.5 Spring

While the spring was the geometry that gave the highest particle residence time in the 1 m section of the reactor tube and encouraged a degree of turbulence, its major disadvantage was that most of the flow was directed through the centre of the helix [Figure 7.9 (a)]. Here, target organisms would be shaded to some degree by the insert and would not receive optimum intensities for solar disinfection due to the diminished concentration factor. In this case, the irradiated area included the tube area on the inside of the helix, where the attenuation due to

the Beer-Lambert Law (Eqn. 7.4) would be slightly higher, although still negligible for practical purposes, with a maximum value of 1.9 %. The losses due to the refraction would be greater however and must be taken into consideration. The mixing of the fluid can be seen in the sample particle track displayed in Figure 7.9 (b). The 11.3 % of particles striking the insert is an encouraging return for an insert with such a small volume, but the concentration of the flow through the centre imposes limitations on this design as discussed.



(a)



(b)

Figure 7.9 (a) Contours of velocity magnitude (m/s) for Model 14; (b) sample single particle track for Model 14 (again, the $z = 0$ plane is included for context)

In Model 15, the cylinder with radius 17.29 mm was embedded in the spring from Model 14, in order to investigate whether the mixing promoted by the spring would influence the number of particle hits. This effect was seen to a degree, with 9.8 % of particles trapped as opposed to 8.9 % without the spring. The improvement may have been more significant had the spring been expanded, such that the cylinder was completely inscribed.

7.3.6 Summary of analyses

As cylinder radius increased, the number of particles coming into contact with the inserts increased, which would be expected due to the limited cross-sectional area available for flow. It must be determined from experiments whether the relationship between the number of particle hits and residence time in the vicinity of the insert can be optimised.

Another factor that would have to be investigated in experiments is whether any sloughing of TiO_2 occurs at the high turbulence and velocity of the fluid passing through the reactor.

While frusta displayed favourable characteristics in general over cylindrical inserts, the combination of frusta to create what could be described as a 'Christmas tree' shape, showed the most encouraging results in terms of the numbers of particles coming into contact with the irradiated area. This has interesting implications for the future design of fixed photocatalytic supports for photoreactors, as optical losses due to the change in shape are minimal, as are changes in particle residence times in the insert region. One factor to be considered in these frustum-shaped designs is whether biofilms accumulate at the intersection of adjacent frusta.

A geometry recommended for further modeling is an arrangement of consecutive frusta, in which the first frustum decreases in radius in the direction of flow and the subsequent frusta have radii which increase in the direction of flow. That is, the first frustum in Model 12 would be turned around, with the others remaining as they are, as shown in Figure 7.10. This would combine the turbulence properties found at the frustum intersections and favourable shape seen in Model 12 with the turbulence due to sudden constriction of flow at the start of the insert region, seen in Model 13. This turbulence at the beginning of the insert region implies that a significant number of particle hits will occur at that point. Therefore, it might be preferable for the insert to be positioned such that there is a short initial flow period in the

irradiated reactor tube before the insert is met. This could be a more effective alternative to a lot of current designs where the insert support spans the length of the irradiated tube. However, fixed inserts have to be held in place in the centre of the reactor tube, so structures for achieving this would have to be considered in further designs.

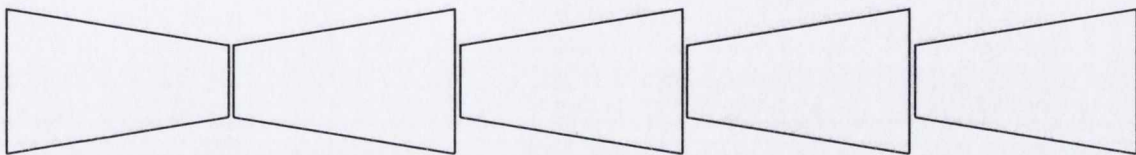


Figure 7.10 Sketch of possible future design

Clearly, modelling alone is insufficient to determine the exact disinfection capabilities of a particular insert geometry. Therefore it is planned in further research to conduct experimental analyses of the inserts simulated in this chapter. The modelling carried out utilised a relatively simplistic approach, with a relatively low number of particles considered, no investigation of cell/catalyst surface charge effects and simplified incident intensity assumptions. There was also no in-depth analysis into where exactly on the irradiated area particle interactions occur, if all particles are incident on one point, disinfection would be limited by the production of radicals at that one point. However, the comparative efficiencies of the various inserts provide interesting possibilities for future fixed photocatalytic designs as it is hoped that the results seen in this chapter can be correlated with real disinfection kinetics.

8. CONCLUSIONS

8.1 Conclusions of Research

8.1.1 Post-irradiation effects of photolysis and photocatalysis

In the bench-scale reactor, photocatalysis has been shown to be an effective method of inactivating *E. coli* under relatively low light intensity - average values ranged from 15 to 33 W/m² - at a concentration of 50 mg/L, meaning that potentially it could also have applications in climates where intense sunlight is less abundant. Inactivation of bacteria with 50 mg/L of suspended TiO₂ photocatalyst was far more efficient than when no catalyst was used. When a catalyst was added, the initial inactivation rate was four times higher under artificial UV when compared to inactivation rates in the absence of catalyst.

The phenomenon of post-irradiation inactivation was more clearly seen in the presence of a photocatalyst, compared to photolytic experiments where viable cells were still counted 48 hours after photolytic treatment had been undertaken for most of the samples. After 15 minutes of exposure to sunlight in the presence of a photocatalyst complete cell destruction 24 and 48 hours post-irradiation was achieved, i.e. the EDT_{48h} were far lower when 50 mg/L TiO₂ was used, compared with those in the absence of catalyst. In some cases in the photolytic experiments, the EDT was not achieved despite six hours of illumination. This does not necessarily mean that solar disinfection does not have a residual effect on bacteria post-irradiation, indeed it has been proven to at high enough a dose (Berney *et al.*, 2006a), but its effect is far less pronounced than that of solar photocatalytic disinfection.

A full-scale solar photocatalytic treatment system would require a reservoir to store the treated water before consumption and these findings imply that bacterial inactivation would continue in such a reservoir. It must be noted, however, that the experiments outlined above were carried out in sterile water and not in more realistic surface or ground water quality conditions where water is typically sourced. To this end, further testing was carried out on distilled water, river water and rainwater in the full-scale CPC under. When river water was used, regrowth was seen in samples even after they had reached the MDL in the absence of catalyst. However,

when TiO_2 was introduced, bacterial die-off continued in the dark, albeit with a higher UV dose required to achieve total inactivation than in rainwater and distilled water.

The tests under sunlight were carried out at a more northerly latitude and in a more temperate climate than most conducted in previous research, yet solar photocatalytic disinfection was still efficient. This would imply that such reactor systems would be suitable not just in tropical and equatorial regions, but in any locations where a moderate amount of sunlight is received.

8.1.2 The effect of stroboscopic light patterns on bacterial inactivation

It was seen that during photolytic experiments, introducing more frequent pulses of exposure to UV illumination while keeping the irradiated volume the same was seen to have little effect on disinfection. In photocatalytic tests, however, the general trend was that configurations with more illumination interruptions led to higher disinfection rates and shorter EDTs. The optimal configuration with one pipe area performed as effectively as the six pipe configuration. It is hypothesised that direct mechanisms are the main cause of UV disinfection and that these require the bacteria to be irradiated for longer periods of time for cells to be killed efficiently. Alternatively, meanwhile, photocatalysis proceeds primarily via indirect mechanisms involving the creation of reactive oxygen species. These may be produced and reacted more efficiently when spread out over more, shorter time periods. Unless similar illumination patterns are used, cumulative dose has been shown to be a poor parameter by which to compare disinfection rates in photolysis and photocatalysis, which reflects previous findings (Rincón & Pulgarin, 2004b).

From an engineering design perspective, the author would recommend that the above findings could lead to smaller, more efficient reactor configurations. The implications that more interruptions improve photocatalytic disinfection could be implemented by introducing light and dark periods into photocatalytic reactor systems, subject to further testing. It also suggests that the introduction of fixed catalysts may have an extra benefit in this regard, as microorganisms would be subjected to a higher frequency of dark and light periods.

8.1.3 The disinfection of phages

The bacteriophages studied, MS2, Φ X174 and PR772, showed varying disinfection characteristics under UV and solar treatment. Under artificial UV, the resistance of phages to both photolytic and artificial treatment was found to decrease in the following order: PR772> Φ X174>MS2. Under solar treatment, PR772 was inactivated far more readily and it is thought that the variation in solar intensity during the trials had a major effect on disinfection rates, as kinetics for phages were quite different under different weather conditions.

The use of a range of indicator organisms is suggested when studying solar and solar photocatalytic disinfection, as each the relative response of each organism to different experimental conditions has shown large variation.

In the development a reactor system, the engineer should take the disinfection rates of a number of different relevant viruses and design the system to disinfect the most resistant.

8.1.4 The modelling of possible insert geometries

As might be expected, as the radius of the hypothetical cylindrical insert was increased, the percentage of modelled *E. coli* cells coming into contact with the insert also increased. However, this comes at the cost of a decrease in particle residence time in the vicinity of the insert, meaning cell/catalyst particle interactions may be less favourable for disinfection.

The use of a frustum brought about a far greater number of particle hits than a cylinder with a similar volume and irradiated surface area, especially when the radius increased with the direction of flow. An arrangement of consecutive frusta was seen to improve performance further and was the most efficient design of those studied.

An experimental study of the various insert designs is planned in order to correlate the findings of Chapter 7 with actual inactivation rates. These experiments will have to take into account the influence of factors such as cell residence time, catalyst sloughing and mechanical stresses imposed by the changing fluid velocities.

8.1.5 Summary of Conclusions

In studies into bacterial inactivation rates, post-irradiation effects and viral disinfection, solar photocatalysis using suspended TiO_2 repeatedly showed better results than solar photolysis. It was proven to be an efficient method of disinfecting *E. coli* and a number of bacteriophages, even at a northerly latitude. It is hoped that if more efficient support geometries for fixed photocatalysis can be created and implemented in solar CPC reactors, they will provide a sustainable method of drinking water treatment in developing countries. The benefits of such a system would include the possible prevention of bacterial recovery post-treatment, low maintenance costs and high disinfection rates.

8.2 Recommendations for Further Research

- The use of distilled water in solar and solar photocatalysis should be limited, with natural waters more appropriate to determine disinfection characteristics in practical applications.
- When using different reactor systems, although results will show the same general trends, direct up-scaling of experimental results is not recommended, due to factors such as the time spent in the dark per traverse and the length of each illumination period.
- Further microscopic investigations to better determine the mechanism of cell death by photolysis and photocatalysis would be recommended.
- The introduction of stroboscopic light conditions in solar photocatalytic reactors might allow for the construction of more efficient, smaller reactor configurations. These could be incorporated in possible fixed catalyst support designs.
- The use of a number of organisms in solar and solar photocatalytic testing is recommended, due to the different relative responses of organisms to different experimental conditions.
- The investigation of disinfection of phages in a CPC with supported photocatalyst is recommended, as research has shown that, in some cases, phage/ TiO_2 particle interactions are not favoured due to surface charge (Cho *et al.*, 2005).

- More research into alternative insert geometries is recommended, which should be undertaken in conjunction with experimental testing of the designed shapes.
- The influence of particle velocity near an insert surface needs to be researched for further optimisation of design.
- One such geometry recommended for modeling is an arrangement of consecutive frusta, in which the first frustum decreases in radius in the direction of flow and the subsequent frusta have radii which increase in the direction of flow.

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APPENDIX A

Date: 18/06/2008
Bacteria: E. Coli K-12
Reactor: Small CPC
Catalyst: 50mg/l TiO2

Time (min)	Dilution						
	0'	0	-1	-2	-3		
0							
15			21	21	37	42	
30		43	24				
45		30	21				
60		20	23				
90		12	9				
120	1						
180	1						
240	0						
300	0						
360	0						

Time (min)	Dilution							CFU/ml	N/N ₀	log(N/N ₀)
	0'	0	-1	-2	-3					
0				185000	210000			197500	1	0
15			10500	10500				10500	0.053165	-1.27438
30		2150	1200					1675	0.008481	-2.07155
45		1500	1050					1275	0.006456	-2.19006
60		1000	1150					1075	0.005443	-2.26416
90		600	450					525	0.002658	-2.57541
120	5							50	0.000253	-3.5966
180	5							50	0.000253	-3.5966
240	0							50	0.000253	-3.5966
300	0							50	0.000253	-3.5966
360	0							50	0.000253	-3.5966

After 2 hours

Time (min)	Dilution						
	0'	0	-1	-2	-3		
0							
15		25	25	10	20		
30	15						
45	0						
60	0						
90	40						
120	0						
180	0						
240	0						
300	0						
360	0						

Time (min)	Dilution							CFU/ml
	0'	0	-1	-2	-3			
0				50000	100000			75000
15		1250	1250					1250
30	15	0	0					75
45	0	0	0					50
60	0	0	0					50
90	200	0	0					200
120	1	0	0					50
180	0	0	0					50
240	0	0	0					50
300	0	0	0					50
360	0	0	0					50

After 24 hours

Time (min)	Dilution						
	0'	0	-1	-2	-3		
0			13	11			
15	0						
30		43	47				
45	0						
60	0						
90	0						
120	0						
180	0						
240	0						
300	0						
360	0						

Time (min)	Dilution							CFU/ml
	0'	0	-1	-2	-3			
0			6500	5500				6000
15	0		0	0	x	x	x	50
30		2150	2350	0	0	x	x	2250
45	0	0	0	0	0	x	x	50
60	0	0	0	0	0	x	x	50
90	0	0	0	0	0	x	x	50
120	0	0	0	0	0	x	x	50
180	0	0	0	0	0	x	x	50
240	0	0	0	0	0	x	x	50
300	0	0	0	0	0	x	x	50
360	0	0	0	0	0	x	x	50

After 48 hours

Time (min)	Dilution						
	0'	0	-1	-2	-3		
0			5	10			
15	0						
30	0						
45	0						
60	0						
90	0						
120	0						
180	0						
240	0						
300	0						
360	0						

Time (min)	Dilution							CFU/ml
	0'	0	-1	-2	-3			
0			2500	5000				3750
15	0							50
30	0							50
45	0							50
60	0							50
90	0							50
120	0							50
180	0							50
240	0							50
300	0							50
360	0							50

Date: 17/08/2009
 Virus: PR772
 Reactor: Large CPC
 Catalyst: 0mg/l TiO2

Time (min)	Dilution						
	0'	0	-1	-2	-3	-4	
0					112	108	
15					43	47	
30					42	41	
45					38	33	
60					33	21	
90					12	12	
120			60	50			
180		9	8				
240	6						
300	5						
360	4						

Time (min)	Dilution							PFU/ml	N/No	log(N/No)	Timestep UV Dose	Cumulative UV
	0'	0	-1	-2	-3	-4						
0					5600000	5400000		5500000	1	0	0	0
15					2150000	2350000		2250000	0.409090909	-0.38818	0.862474	0.862474
30					2100000	2050000		2075000	0.377272727	-0.42334	2.428382	3.290856
45					1900000	1650000		1775000	0.322727273	-0.49116	2.272564	5.56342
60					1650000	1050000		1350000	0.245454545	-0.61003	2.652291	8.215711
90					600000	600000		600000	0.109090909	-0.96221	4.672753	12.88846
120			30000	25000				27500	0.005	-2.30103	2.722655	15.61112
180		450	400					425	7.72727E-05	-4.11197	1.895566	17.50668
240	30							50	9.09091E-06	-5.04139	0.40176	17.90844
300	25							50	9.09091E-06	-5.04139	0.36725	18.27569
360	20							50	9.09091E-06	-5.04139	0.325625	18.60132

Date: #####
Virus: Large CPC
Reactor: 50mg/l TiO2
Catalyst:

Time (min)	Dilution							
	0'	0	-1	-2	-3	-4		
0								
15				192	180			
30			25	33				
45		6	6					
60	0							
90	0							
120	0							
180	0							
240	0							
300	0							
360	0							

Time (min)	Dilution								PFU/ml	N/No	log(N/No)	Timestep UV Dose	Cumulative e UV
	0'	0	-1	-2	-3	-4							
0	0	0	0	0	0	0	0	0	4500000	1	0	0	0
15	0	0	0	0	0	0	0	0	930000	0.206667	-0.68473	1.83863	1.83863
30	0	0	0	0	0	0	0	0	14500	0.003222	-2.49184	1.9796	3.81823
45	0	300	300	0	0	0	0	0	300	6.67E-05	-4.17609	1.883008	5.701238
60	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	1.766481	7.467718
90	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	3.928658	11.39638
120	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	4.676973	16.07335
180	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	4.329636	20.40299
240	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	3.027284	23.43027
300	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	3.646865	27.07713
360	0	0	0	0	0	0	0	0	50	1.11E-05	-4.95424	1.89093	28.96806

Time	UV-A Intensity [mW/cm²]	UV-A Intensity [W/m²]	UV-B Intensity [µW/cm²]	UV-B Intensity [W/m²]	Combined UV Intensity [W/m²]	Sample Time	Timestep average UV dose [W/m²]	Cumulative Dose over timestep [kJ/L]	Cumulative Dose [kJ/L]
10:57:00	2.27	22.7	3.71	0.0371	22.7371	0	0	0	0
10:58:00	2.3	23	3.76	0.0376	23.0376	1			
10:59:00	2.312	23.12	3.882	0.03882	23.15882	2			
11:00:00	2.31	23.1	3.96	0.0396	23.1396	3			
11:01:00	2.372	23.72	3.95	0.0395	23.7595	4			
11:02:00	2.393	23.93	4.133	0.04133	23.97133	5			
11:03:00	2.413	24.13	4.22	0.0422	24.1722	6			
11:04:00	2.424	24.24	4.17	0.0417	24.2817	7			
11:05:00	2.48	24.8	4.37	0.0437	24.8437	8			
11:06:00	2.483	24.83	4.173	0.04173	24.87173	9			
11:07:00	2.45	24.5	4.482	0.04482	24.54482	10			
11:08:00	2.53	25.3	4.391	0.04391	25.34391	11			
11:09:00	2.491	24.91	4.521	0.04521	24.95521	12			
11:10:00	2.572	25.72	4.59	0.0459	25.7659	13			
11:11:00	2.584	25.84	4.64	0.0464	25.8864	14			
11:12:00	2.59	25.9	4.592	0.04592	25.94592	15	24.51189	1.592507	1.592507
11:13:00	2.62	26.2	4.712	0.04712	26.24712	16			
11:14:00	2.62	26.2	4.82	0.0482	26.2482	17			
11:15:00	2.63	26.3	4.79	0.0479	26.3479	18			
11:16:00	2.64	26.4	4.8	0.048	26.448	19			
11:17:00	2.66	26.6	4.924	0.04924	26.64924	20			
11:18:00	2.68	26.8	4.88	0.0488	26.8488	21			
11:19:00	2.72	27.2	5.06	0.0506	27.2506	22			
11:20:00	2.73	27.3	5.04	0.0504	27.3504	23			
11:21:00	2.733	27.33	5.1	0.051	27.381	24			
11:22:00	2.63	26.3	4.96	0.0496	26.3496	25			
11:23:00	2.77	27.7	5.17	0.0517	27.7517	26			
11:24:00	2.75	27.5	5.093	0.05093	27.55093	27			
11:25:00	2.722	27.22	5.35	0.0535	27.2735	28			
11:26:00	2.751	27.51	5.242	0.05242	27.56242	29			
11:27:00	2.814	28.14	5.27	0.0527	28.1927	30	27.03014	1.756114	3.348621
11:28:00	2.844	28.44	5.34	0.0534	28.4934	31			
11:29:00	2.84	28.4	5.38	0.0538	28.4538	32			
11:30:00	2.89	28.9	5.594	0.05594	28.95594	33			
11:31:00	2.6	26	5.53	0.0553	26.0553	34			
11:32:00	2.94	29.4	5.261	0.05261	29.45261	35			
11:33:00	2.294	22.94	5.49	0.0549	22.9949	36			
11:34:00	2.844	28.44	4.9	0.049	28.489	37			
11:35:00	1.922	19.22	4.434	0.04434	19.26434	38			
11:36:00	3.084	30.84	4.084	0.04084	30.88084	39			
11:37:00	3.02	30.2	5.83	0.0583	30.2583	40			
11:38:00	3.061	30.61	5.8	0.058	30.668	41			
11:39:00	2.803	28.03	5.954	0.05954	28.08954	42			
11:40:00	3.09	30.9	5.92	0.0592	30.9592	43			
11:41:00	3.1	31	6.05	0.0605	31.0605	44			
11:42:00	3.09	30.9	6.04	0.0604	30.9604	45	28.33574	1.840937	5.189559
11:43:00	3.092	30.92	6.2	0.062	30.982	46			
11:44:00	2.501	25.01	6.042	0.06042	25.07042	47			
11:45:00	2.42	24.2	5.24	0.0524	24.2524	48			
11:46:00	3.21	32.1	6.13	0.0613	32.1613	49			
11:47:00	3.25	32.5	6.32	0.0632	32.5632	50			
11:48:00	1.93	19.3	5.754	0.05754	19.35754	51			
11:49:00	3.26	32.6	6.124	0.06124	32.66124	52			
11:50:00	3.26	32.6	6.34	0.0634	32.6634	53			
11:51:00	2.93	29.3	6.05	0.0605	29.3605	54			
11:52:00	3.172	31.72	6.102	0.06102	31.78102	55			
11:53:00	3.29	32.9	6.36	0.0636	32.9636	56			
11:54:00	3.35	33.5	6.561	0.06561	33.56561	57			
11:55:00	3.37	33.7	6.64	0.0664	33.7664	58			
11:56:00	3.3	33	6.643	0.06643	33.06643	59			

Sample analysis of UV data for experiment under natural sunlight

11:57:00	3.183	31.83	6.59	0.0659	31.8959	60	30.4074	1.975531	7.165089
11:58:00	3.29	32.9	6.601	0.06601	32.96601	61			
11:59:00	3.15	31.5	6.57	0.0657	31.5657	62			
12:00:00	2.73	27.3	5.9	0.059	27.359	63			
12:01:00	3.18	31.8	6.18	0.0618	31.8618	64			
12:02:00	3.18	31.8	6.34	0.0634	31.8634	65			
12:03:00	3.281	32.81	6.641	0.06641	32.87641	66			
12:04:00	3.45	34.5	6.801	0.06801	34.56801	67			
12:05:00	3.483	34.83	7.01	0.0701	34.9001	68			
12:06:00	2.95	29.5	7.04	0.0704	29.5704	69			
12:07:00	3.52	35.2	6.983	0.06983	35.26983	70			
12:08:00	3.534	35.34	7.144	0.07144	35.41144	71			
12:09:00	3.54	35.4	7.25	0.0725	35.4725	72			
12:10:00	3.58	35.8	7.282	0.07282	35.87282	73			
12:11:00	3.59	35.9	7.34	0.0734	35.9734	74			
12:12:00	3.6	36	7.4	0.074	36.074	75			
12:13:00	3.62	36.2	7.42	0.0742	36.2742	76			
12:14:00	3.59	35.9	7.47	0.0747	35.9747	77			
12:15:00	3.593	35.93	7.431	0.07431	36.00431	78			
12:16:00	3.6	36	7.35	0.0735	36.0735	79			
12:17:00	3.59	35.9	7.512	0.07512	35.97512	80			
12:18:00	3.622	36.22	7.531	0.07531	36.29531	81			
12:19:00	3.641	36.41	7.61	0.0761	36.4861	82			
12:20:00	3.652	36.52	7.592	0.07592	36.59592	83			
12:21:00	3.68	36.8	7.563	0.07563	36.87563	84			
12:22:00	3.721	37.21	7.712	0.07712	37.28712	85			
12:23:00	3.7	37	7.73	0.0773	37.0773	86			
12:24:00	3.583	35.83	7.753	0.07753	35.90753	87			
12:25:00	3.452	34.52	7.78	0.0778	34.5978	88			
12:26:00	3.66	36.6	7.714	0.07714	36.67714	89			
12:27:00	3.671	36.71	7.684	0.07684	36.78684	90	34.88311	4.532624	11.69771
12:28:00	3.714	37.14	7.884	0.07884	37.21884	91			
12:29:00	3.73	37.3	7.86	0.0786	37.3786	92			
12:30:00	3.684	36.84	8	0.08	36.92	93			
12:31:00	3.753	37.53	7.88	0.0788	37.6088	94			
12:32:00	3.68	36.8	7.92	0.0792	36.8792	95			
12:33:00	3.79	37.9	7.991	0.07991	37.97991	96			
12:34:00	3.82	38.2	8.09	0.0809	38.2809	97			
12:35:00	3.752	37.52	8.1	0.081	37.601	98			
12:36:00	3.78	37.8	8.144	0.08144	37.88144	99			
12:37:00	3.781	37.81	8.262	0.08262	37.89262	100			
12:38:00	3.81	38.1	8.171	0.08171	38.18171	101			
12:39:00	3.831	38.31	8.142	0.08142	38.39142	102			
12:40:00	3.872	38.72	8.33	0.0833	38.8033	103			
12:41:00	3.89	38.9	8.41	0.0841	38.9841	104			
12:42:00	3.861	38.61	8.443	0.08443	38.69443	105			
12:43:00	3.874	38.74	8.45	0.0845	38.8245	106			
12:44:00	3.89	38.9	8.43	0.0843	38.9843	107			
12:45:00	3.892	38.92	8.384	0.08384	39.00384	108			
12:46:00	3.8	38	8.423	0.08423	38.08423	109			
12:47:00	3.86	38.6	8.52	0.0852	38.6852	110			
12:48:00	3.881	38.81	8.51	0.0851	38.8951	111			
12:49:00	3.86	38.6	8.27	0.0827	38.6827	112			
12:50:00	3.863	38.63	8.431	0.08431	38.71431	113			
12:51:00	3.871	38.71	8.27	0.0827	38.7927	114			
12:52:00	3.89	38.9	8.584	0.08584	38.98584	115			
12:53:00	3.893	38.93	8.53	0.0853	39.0153	116			
12:54:00	3.87	38.7	8.501	0.08501	38.78501	117			
12:55:00	3.923	39.23	8.34	0.0834	39.3134	118			
12:56:00	3.94	39.4	8.82	0.0882	39.4882	119			
12:57:00	3.942	39.42	8.63	0.0863	39.5063	120	38.41524	4.99158	16.68929
12:58:00	3.943	39.43	8.94	0.0894	39.5194	121			
12:59:00	3.94	39.4	8.341	0.08341	39.48341	122			

Sample analysis of UV data for experiment under natural sunlight

13:00:00	3.991	39.91	8.83	0.0883	39.9983	123			
13:01:00	4	40	8.42	0.0842	40.0842	124			
13:02:00	4.01	40.1	8.94	0.0894	40.1894	125			
13:03:00	4.02	40.2	8.772	0.08772	40.28772	126			
13:04:00	4.031	40.31	8.71	0.0871	40.3971	127			
13:05:00	4.03	40.3	8.84	0.0884	40.3884	128			
13:06:00	4.05	40.5	8.7	0.087	40.587	129			
13:07:00	4.04	40.4	9.001	0.09001	40.49001	130			
13:08:00	4.044	40.44	8.511	0.08511	40.52511	131			
13:09:00	4.04	40.4	9.013	0.09013	40.49013	132			
13:10:00	4.043	40.43	8.683	0.08683	40.51683	133			
13:11:00	4.044	40.44	9.363	0.09363	40.53363	134			
13:12:00	4.053	40.53	9.23	0.0923	40.6223	135			
13:13:00	4.06	40.6	8.23	0.0823	40.6823	136			
13:14:00	4.08	40.8	8.562	0.08562	40.88562	137			
13:15:00	4.08	40.8	9.574	0.09574	40.89574	138			
13:16:00	4.09	40.9	7.84	0.0784	40.9784	139			
13:17:00	4.07	40.7	9.01	0.0901	40.7901	140			
13:18:00	4.06	40.6	9.214	0.09214	40.69214	141			
13:19:00	4.06	40.6	8.9	0.089	40.689	142			
13:20:00	4.054	40.54	10.31	0.1031	40.6431	143			
13:21:00	4.08	40.8	10.04	0.1004	40.9004	144			
13:22:00	4.084	40.84	10.2	0.102	40.942	145			
13:23:00	4.082	40.82	7.85	0.0785	40.8985	146			
13:24:00	4.092	40.92	9.01	0.0901	41.0101	147			
13:25:00	4.084	40.84	8.77	0.0877	40.9277	148			
13:26:00	4.12	41.2	8.77	0.0877	41.2877	149			
13:27:00	4.08	40.8	9.59	0.0959	40.8959	150			
13:28:00	4.1	41	8.351	0.08351	41.08351	151			
13:29:00	4.092	40.92	9.66	0.0966	41.0166	152			
13:30:00	4.113	41.13	8.941	0.08941	41.21941	153			
13:31:00	4.124	41.24	7.97	0.0797	41.3197	154			
13:32:00	4.11	41.1	9.87	0.0987	41.1987	155			
13:33:00	4.1	41	8.183	0.08183	41.08183	156			
13:34:00	4.084	40.84	9.4	0.094	40.934	157			
13:35:00	4.153	41.53	9.28	0.0928	41.6228	158			
13:36:00	3.9	39	8.37	0.0837	39.0837	159			
13:37:00	4.173	41.73	9.17	0.0917	41.8217	160			
13:38:00	4.16	41.6	8.932	0.08932	41.68932	161			
13:39:00	4.111	41.11	8.843	0.08843	41.19843	162			
13:40:00	4.153	41.53	9.164	0.09164	41.62164	163			
13:41:00	4.16	41.6	8.924	0.08924	41.68924	164			
13:42:00	4.163	41.63	9.02	0.0902	41.7202	165			
13:43:00	4.103	41.03	8.94	0.0894	41.1194	166			
13:44:00	4.124	41.24	9.012	0.09012	41.33012	167			
13:45:00	4.07	40.7	8.953	0.08953	40.78953	168			
13:46:00	4.133	41.33	8.672	0.08672	41.41672	169			
13:47:00	4.134	41.34	9.01	0.0901	41.4301	170			
13:48:00	4.142	41.42	8.7	0.087	41.507	171			
13:49:00	4.15	41.5	9.11	0.0911	41.5911	172			
13:50:00	4.16	41.6	8.722	0.08722	41.68722	173			
13:51:00	4.011	40.11	9	0.09	40.2	174			
13:52:00	4.154	41.54	8.98	0.0898	41.6298	175			
13:53:00	4.16	41.6	8.66	0.0866	41.6866	176			
13:54:00	4.15	41.5	9.26	0.0926	41.5926	177			
13:55:00	4.05	40.5	8.653	0.08653	40.58653	178			
13:56:00	4.112	41.12	8.871	0.08871	41.20871	179			
13:57:00	4.15	41.5	8.83	0.0883	41.5883	180	40.91494	5.316384	22.00568
13:58:00	4.154	41.54	9.05	0.0905	41.6305	181			
13:59:00	4.13	41.3	8.902	0.08902	41.38902	182			
14:00:00	4.12	41.2	8.73	0.0873	41.2873	183			
14:01:00	4.111	41.11	8.83	0.0883	41.1983	184			
14:02:00	4.094	40.94	8.75	0.0875	41.0275	185			

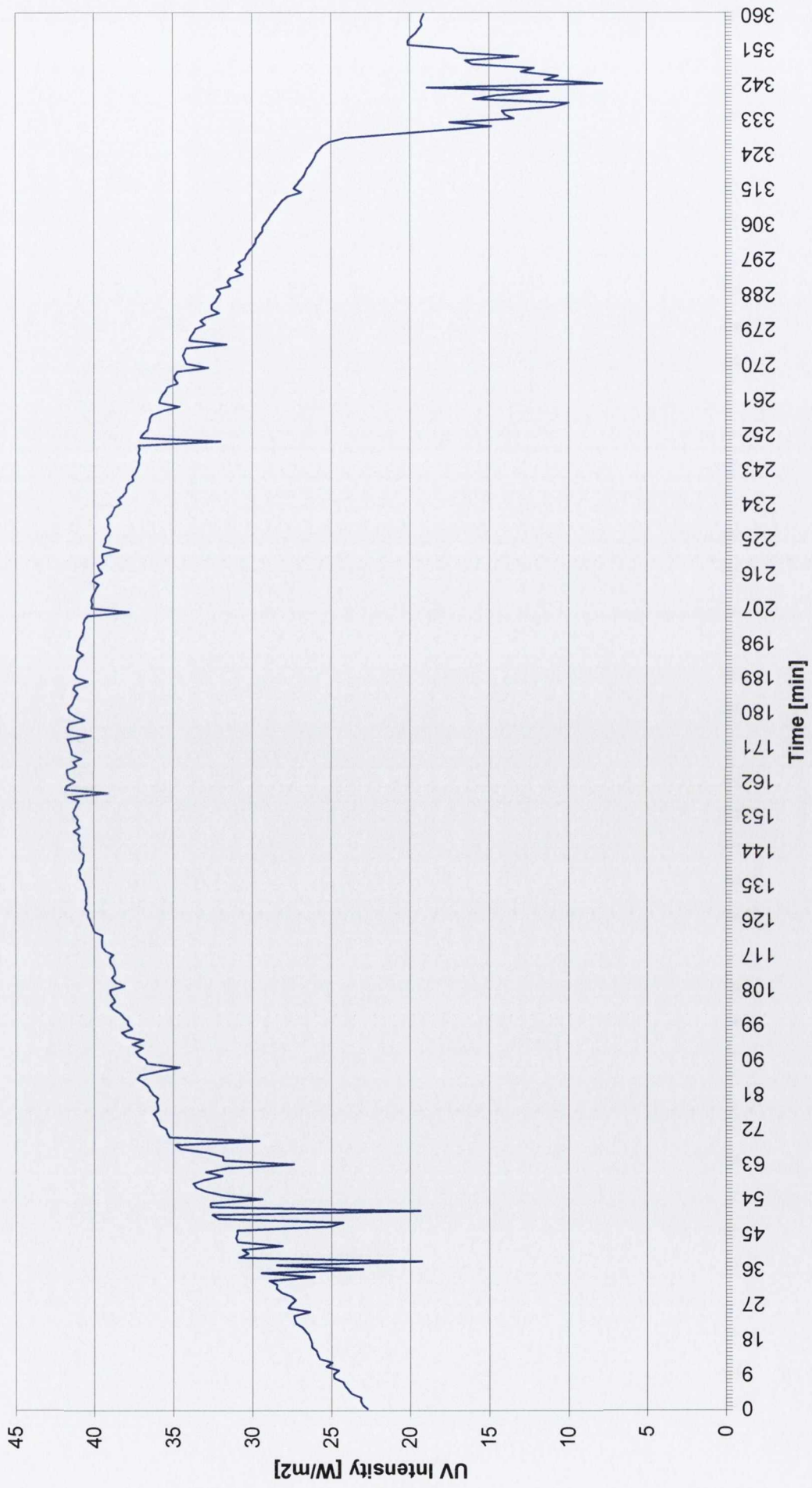
Sample analysis of UV data for experiment under natural sunlight

14:03:00	4.124	41.24	8.63	0.0863	41.3263	186
14:04:00	4.11	41.1	8.87	0.0887	41.1887	187
14:05:00	4.03	40.3	8.77	0.0877	40.3877	188
14:06:00	4.101	41.01	8.5	0.085	41.095	189
14:07:00	4.12	41.2	8.78	0.0878	41.2878	190
14:08:00	4.111	41.11	8.711	0.08711	41.19711	191
14:09:00	4.104	41.04	8.5	0.085	41.125	192
14:10:00	4.102	41.02	8.56	0.0856	41.1056	193
14:11:00	4.09	40.9	8.501	0.08501	40.98501	194
14:12:00	4.06	40.6	8.42	0.0842	40.6842	195
14:13:00	4.1	41	8.532	0.08532	41.08532	196
14:14:00	4.094	40.94	8.59	0.0859	41.0259	197
14:15:00	4.09	40.9	8.74	0.0874	40.9874	198
14:16:00	4.08	40.8	9.55	0.0955	40.8955	199
14:17:00	4.064	40.64	7.99	0.0799	40.7199	200
14:18:00	4.06	40.6	8.032	0.08032	40.68032	201
14:19:00	4.054	40.54	8.94	0.0894	40.6294	202
14:20:00	4.06	40.6	7.71	0.0771	40.6771	203
14:21:00	4.05	40.5	8.361	0.08361	40.58361	204
14:22:00	4.03	40.3	8.531	0.08531	40.38531	205
14:23:00	3.77	37.7	8.102	0.08102	37.78102	206
14:24:00	4.01	40.1	8.34	0.0834	40.1834	207
14:25:00	4.004	40.04	8.21	0.0821	40.1221	208
14:26:00	4.011	40.11	8.27	0.0827	40.1927	209
14:27:00	4.003	40.03	8.303	0.08303	40.11303	210
14:28:00	3.99	39.9	8.1	0.081	39.981	211
14:29:00	3.98	39.8	8.1	0.081	39.881	212
14:30:00	3.96	39.6	8.044	0.08044	39.68044	213
14:31:00	4	40	8.064	0.08064	40.08064	214
14:32:00	3.99	39.9	8.1	0.081	39.981	215
14:33:00	3.94	39.4	8.024	0.08024	39.48024	216
14:34:00	3.96	39.6	8	0.08	39.68	217
14:35:00	3.97	39.7	7.95	0.0795	39.7795	218
14:36:00	3.93	39.3	8.032	0.08032	39.38032	219
14:37:00	3.94	39.4	7.92	0.0792	39.4792	220
14:38:00	3.94	39.4	7.85	0.0785	39.4785	221
14:39:00	3.833	38.33	7.87	0.0787	38.4087	222
14:40:00	3.933	39.33	7.81	0.0781	39.4081	223
14:41:00	3.92	39.2	7.832	0.07832	39.27832	224
14:42:00	3.92	39.2	7.754	0.07754	39.27754	225
14:43:00	3.901	39.01	7.811	0.07811	39.08811	226
14:44:00	3.89	38.9	7.83	0.0783	38.9783	227
14:45:00	3.904	39.04	7.702	0.07702	39.11702	228
14:46:00	3.903	39.03	7.683	0.07683	39.10683	229
14:47:00	3.91	39.1	7.884	0.07884	39.17884	230
14:48:00	3.881	38.81	7.602	0.07602	38.88602	231
14:49:00	3.871	38.71	7.514	0.07514	38.78514	232
14:50:00	3.862	38.62	7.45	0.0745	38.6945	233
14:51:00	3.82	38.2	7.481	0.07481	38.27481	234
14:52:00	3.84	38.4	7.394	0.07394	38.47394	235
14:53:00	3.81	38.1	7.43	0.0743	38.1743	236
14:54:00	3.79	37.9	7.29	0.0729	37.9729	237
14:55:00	3.79	37.9	7.37	0.0737	37.9737	238
14:56:00	3.78	37.8	7.344	0.07344	37.87344	239
14:57:00	3.744	37.44	7.29	0.0729	37.5129	240 39.90537 5.185204 27.19088
14:58:00	3.75	37.5	7.224	0.07224	37.57224	241
14:59:00	3.73	37.3	7.18	0.0718	37.3718	242
15:00:00	3.72	37.2	7.28	0.0728	37.2728	243
15:01:00	3.71	37.1	6.612	0.06612	37.16612	244
15:02:00	3.71	37.1	6.95	0.0695	37.1695	245
15:03:00	3.703	37.03	7.221	0.07221	37.10221	246
15:04:00	3.713	37.13	6.971	0.06971	37.19971	247
15:05:00	3.704	37.04	7.062	0.07062	37.11062	248

15:06:00	3.713	37.13	6.63	0.0663	37.1963	249			
15:07:00	3.19	31.9	7.144	0.07144	31.97144	250			
15:08:00	3.7	37	6.21	0.0621	37.0621	251			
15:09:00	3.68	36.8	7.521	0.07521	36.87521	252			
15:10:00	3.654	36.54	6.29	0.0629	36.6029	253			
15:11:00	3.65	36.5	6.69	0.0669	36.5669	254			
15:12:00	3.642	36.42	6.97	0.0697	36.4897	255			
15:13:00	3.64	36.4	6.691	0.06691	36.46691	256			
15:14:00	3.62	36.2	6.974	0.06974	36.26974	257			
15:15:00	3.55	35.5	6.5	0.065	35.565	258			
15:16:00	3.45	34.5	6.613	0.06613	34.56613	259			
15:17:00	3.582	35.82	6.45	0.0645	35.8845	260			
15:18:00	3.573	35.73	6.36	0.0636	35.7936	261			
15:19:00	3.56	35.6	6.41	0.0641	35.6641	262			
15:20:00	3.54	35.4	6.47	0.0647	35.4647	263			
15:21:00	3.53	35.3	6.261	0.06261	35.36261	264			
15:22:00	3.462	34.62	6.264	0.06264	34.68264	265			
15:23:00	3.483	34.83	6.254	0.06254	34.89254	266			
15:24:00	3.484	34.84	6.16	0.0616	34.9016	267			
15:25:00	3.463	34.63	6.22	0.0622	34.6922	268			
15:26:00	3.27	32.7	6.09	0.0609	32.7609	269			
15:27:00	3.41	34.1	5.92	0.0592	34.1592	270			
15:28:00	3.421	34.21	5.731	0.05731	34.26731	271			
15:29:00	3.432	34.32	5.842	0.05842	34.37842	272			
15:30:00	3.42	34.2	5.962	0.05962	34.25962	273			
15:31:00	3.41	34.1	5.98	0.0598	34.1598	274			
15:32:00	3.154	31.54	5.84	0.0584	31.5984	275			
15:33:00	3.39	33.9	5.872	0.05872	33.95872	276			
15:34:00	3.383	33.83	5.71	0.0571	33.8871	277			
15:35:00	3.364	33.64	5.66	0.0566	33.6966	278			
15:36:00	3.35	33.5	5.622	0.05622	33.55622	279			
15:37:00	3.29	32.9	5.794	0.05794	32.95794	280			
15:38:00	3.32	33.2	5.612	0.05612	33.25612	281			
15:39:00	3.284	32.84	5.61	0.0561	32.8961	282			
15:40:00	3.2	32	5.55	0.0555	32.0555	283			
15:41:00	3.25	32.5	5.4	0.054	32.554	284			
15:42:00	3.25	32.5	5.443	0.05443	32.55443	285			
15:43:00	3.224	32.24	5.373	0.05373	32.29373	286			
15:44:00	3.21	32.1	5.274	0.05274	32.15274	287			
15:45:00	3.194	31.94	5.351	0.05351	31.99351	288			
15:46:00	3.22	32.2	5.2	0.052	32.252	289			
15:47:00	3.131	31.31	5.21	0.0521	31.3621	290			
15:48:00	3.15	31.5	4.98	0.0498	31.5498	291			
15:49:00	3.131	31.31	5.48	0.0548	31.3648	292			
15:50:00	3.051	30.51	4.941	0.04941	30.55941	293			
15:51:00	3.101	31.01	4.46	0.0446	31.0546	294			
15:52:00	3.07	30.7	4.57	0.0457	30.7457	295			
15:53:00	3.08	30.8	5.03	0.0503	30.8503	296			
15:54:00	3.04	30.4	3.884	0.03884	30.43884	297			
15:55:00	3.03	30.3	5.233	0.05233	30.35233	298			
15:56:00	3.013	30.13	3.87	0.0387	30.1687	299			
15:57:00	2.992	29.92	4.53	0.0453	29.9653	300	34.08327	4.428695	31.61958
15:58:00	2.972	29.72	5.644	0.05644	29.77644	301			
15:59:00	2.97	29.7	5.704	0.05704	29.75704	302			
16:00:00	2.95	29.5	5.631	0.05631	29.55631	303			
16:01:00	2.93	29.3	4.96	0.0496	29.3496	304			
16:02:00	2.93	29.3	4.96	0.0496	29.3496	305			
16:03:00	2.912	29.12	4.254	0.04254	29.16254	306			
16:04:00	2.894	28.94	4.373	0.04373	28.98373	307			
16:05:00	2.88	28.8	4.08	0.0408	28.8408	308			
16:06:00	2.86	28.6	4.202	0.04202	28.64202	309			
16:07:00	2.84	28.4	4.16	0.0416	28.4416	310			
16:08:00	2.822	28.22	4.402	0.04402	28.26402	311			

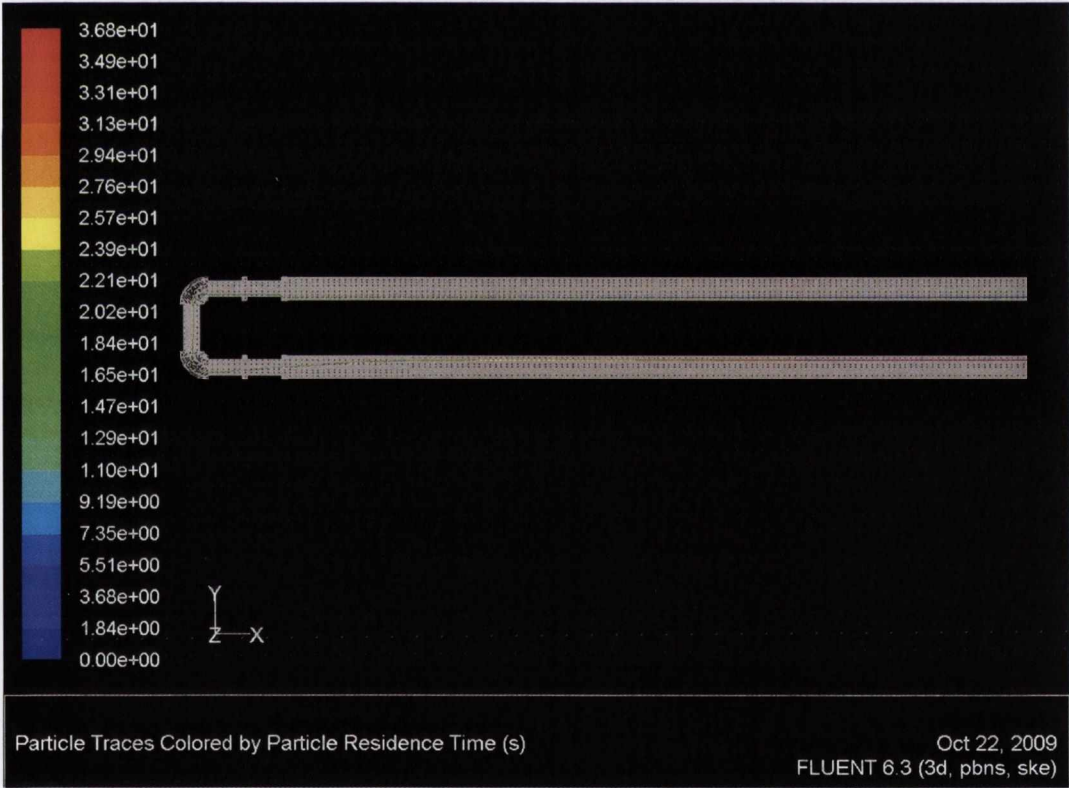
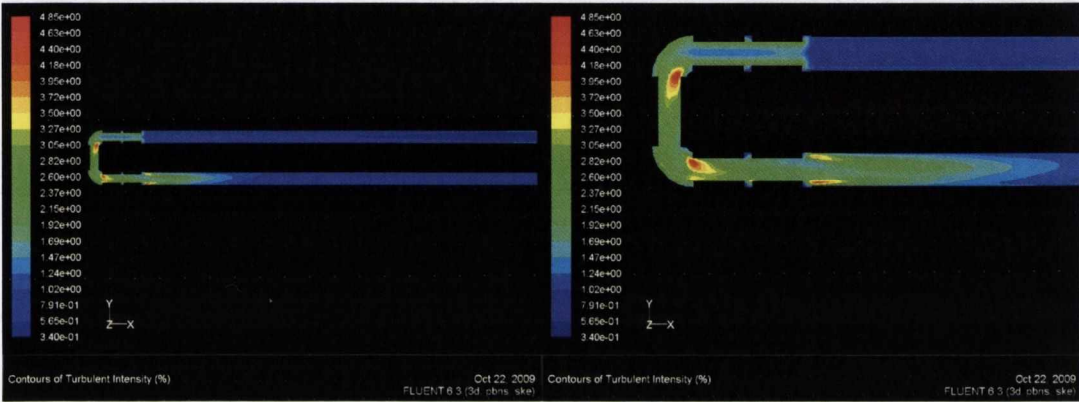
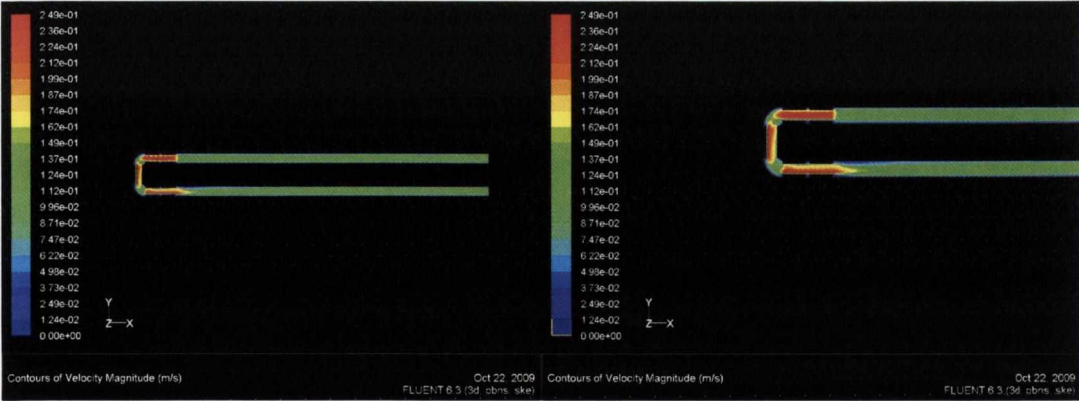
16:09:00	2.8	28	4.39	0.0439	28.0439	312
16:10:00	2.77	27.7	3.98	0.0398	27.7398	313
16:11:00	2.683	26.83	4.284	0.04284	26.87284	314
16:12:00	2.733	27.33	3.94	0.0394	27.3694	315
16:13:00	2.71	27.1	4.033	0.04033	27.14033	316
16:14:00	2.694	26.94	3.73	0.0373	26.9773	317
16:15:00	2.671	26.71	3.88	0.0388	26.7488	318
16:16:00	2.66	26.6	3.8	0.038	26.638	319
16:17:00	2.65	26.5	3.79	0.0379	26.5379	320
16:18:00	2.63	26.3	3.73	0.0373	26.3373	321
16:19:00	2.613	26.13	3.56	0.0356	26.1656	322
16:20:00	2.6	26	3.56	0.0356	26.0356	323
16:21:00	2.592	25.92	2.99	0.0299	25.9499	324
16:22:00	2.57	25.7	3.204	0.03204	25.73204	325
16:23:00	2.55	25.5	2.541	0.02541	25.52541	326
16:24:00	2.51	25.1	2.97	0.0297	25.1297	327
16:25:00	2.414	24.14	2.491	0.02491	24.16491	328
16:26:00	2.112	21.12	2.444	0.02444	21.14444	329
16:27:00	1.794	17.94	2.94	0.0294	17.9694	330
16:28:00	1.482	14.82	2.722	0.02722	14.84722	331
16:29:00	1.75	17.5	1.401	0.01401	17.51401	332
16:30:00	1.34	13.4	1.19	0.0119	13.4119	333
16:31:00	1.4	14	1.97	0.0197	14.0197	334
16:32:00	1.42	14.2	1.794	0.01794	14.21794	335
16:33:00	1.091	10.91	1.542	0.01542	10.92542	336
16:34:00	0.9944	9.944	1.79	0.0179	9.9619	337
16:35:00	1.6	16	1.65	0.0165	16.0165	338
16:36:00	1.45	14.5	2.22	0.0222	14.5222	339
16:37:00	1.12	11.2	1.433	0.01433	11.21433	340
16:38:00	1.891	18.91	2.453	0.02453	18.93453	341
16:39:00	0.7804	7.804	1.19	0.0119	7.8159	342
16:40:00	1.15	11.5	2.052	0.02052	11.52052	343
16:41:00	1.06	10.6	2.853	0.02853	10.62853	344
16:42:00	1.3	13	1.231	0.01231	13.01231	345
16:43:00	1.213	12.13	2.11	0.0211	12.1511	346
16:44:00	1.61	16.1	1.91	0.0191	16.1191	347
16:45:00	1.65	16.5	2.714	0.02714	16.52714	348
16:46:00	1.31	13.1	1.75	0.0175	13.1175	349
16:47:00	1.69	16.9	3.02	0.0302	16.9302	350
16:48:00	1.73	17.3	2.19	0.0219	17.3219	351
16:49:00	2.01	20.1	2.26	0.0226	20.1226	352
16:50:00	2.01	20.1	2.45	0.0245	20.1245	353
16:51:00	1.99	19.9	2.35	0.0235	19.9235	354
16:52:00	1.96	19.6	2.29	0.0229	19.6229	355
16:53:00	1.942	19.42	2.02	0.0202	19.4402	356
16:54:00	1.922	19.22	2.121	0.02121	19.24121	357
16:55:00	1.93	19.3	2.152	0.02152	19.32152	358
16:56:00	1.92	19.2	2.41	0.0241	19.2241	359
16:57:00	1.91	19.1	1.87	0.0187	19.1187	360 21.25359 2.761638 34.38122

Plot of UV Intensity vs Time

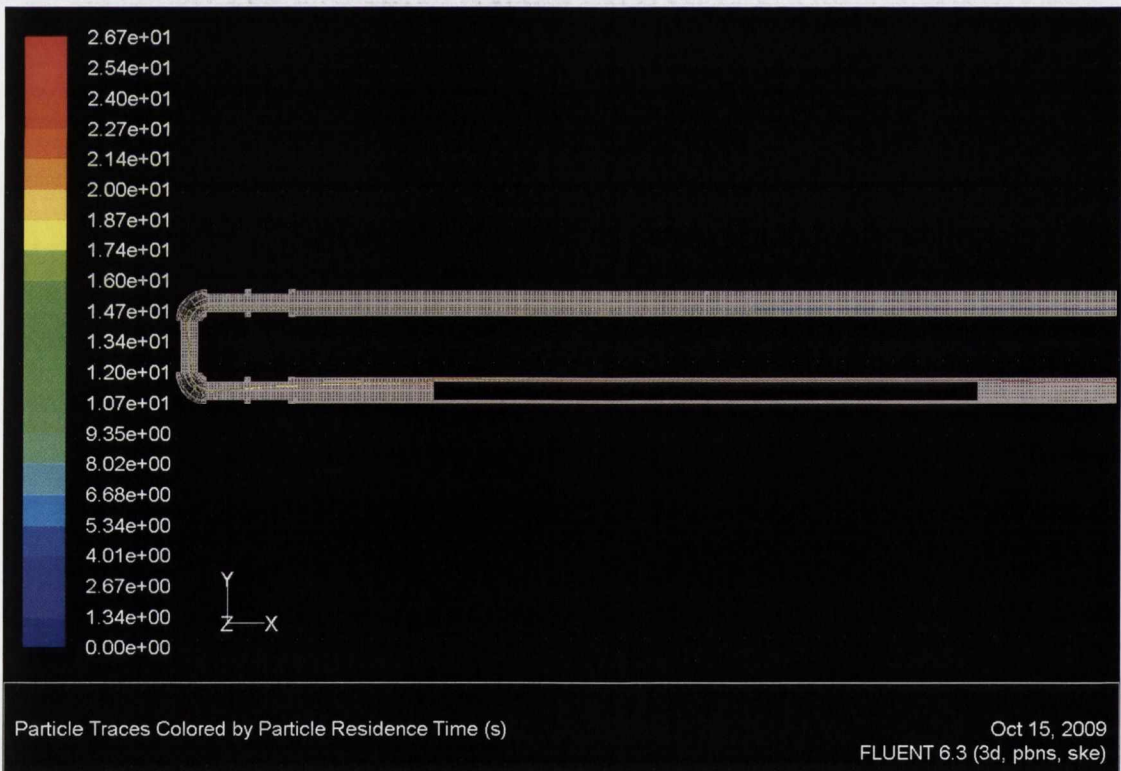
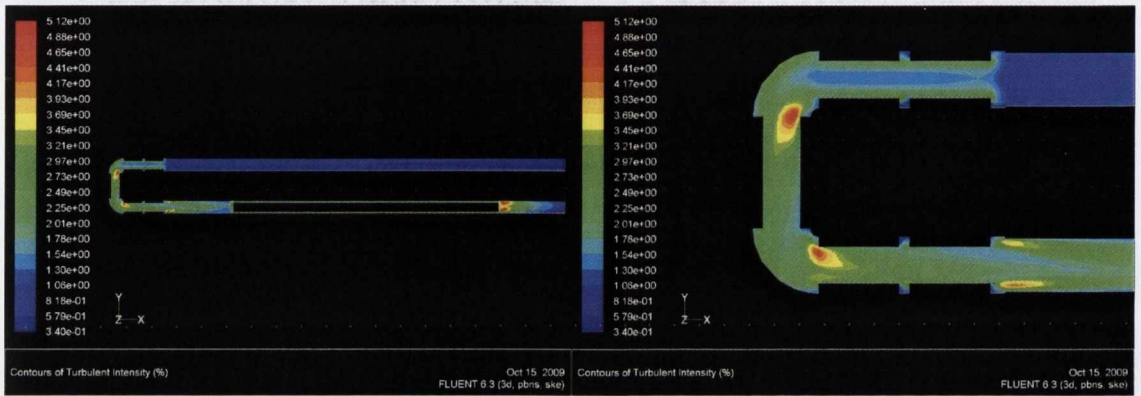
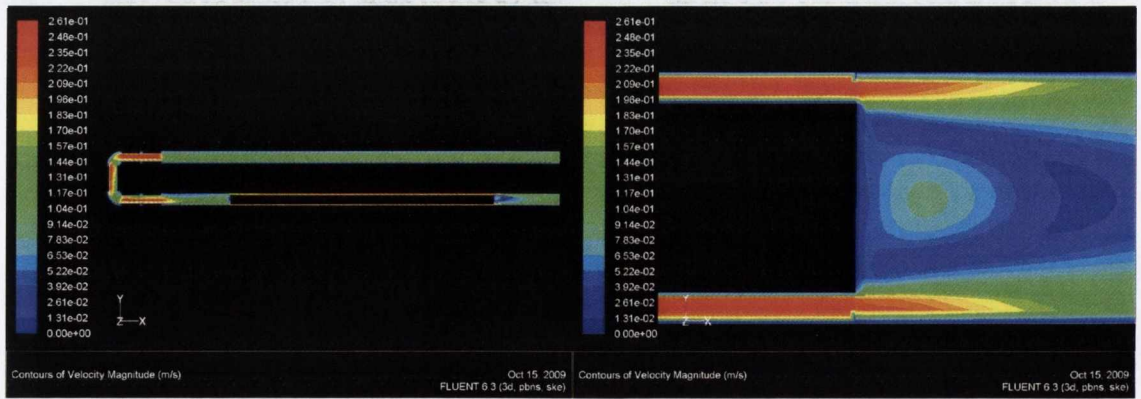


Sample plot of UV Intensity vs Time during an experiment under natural sunlight

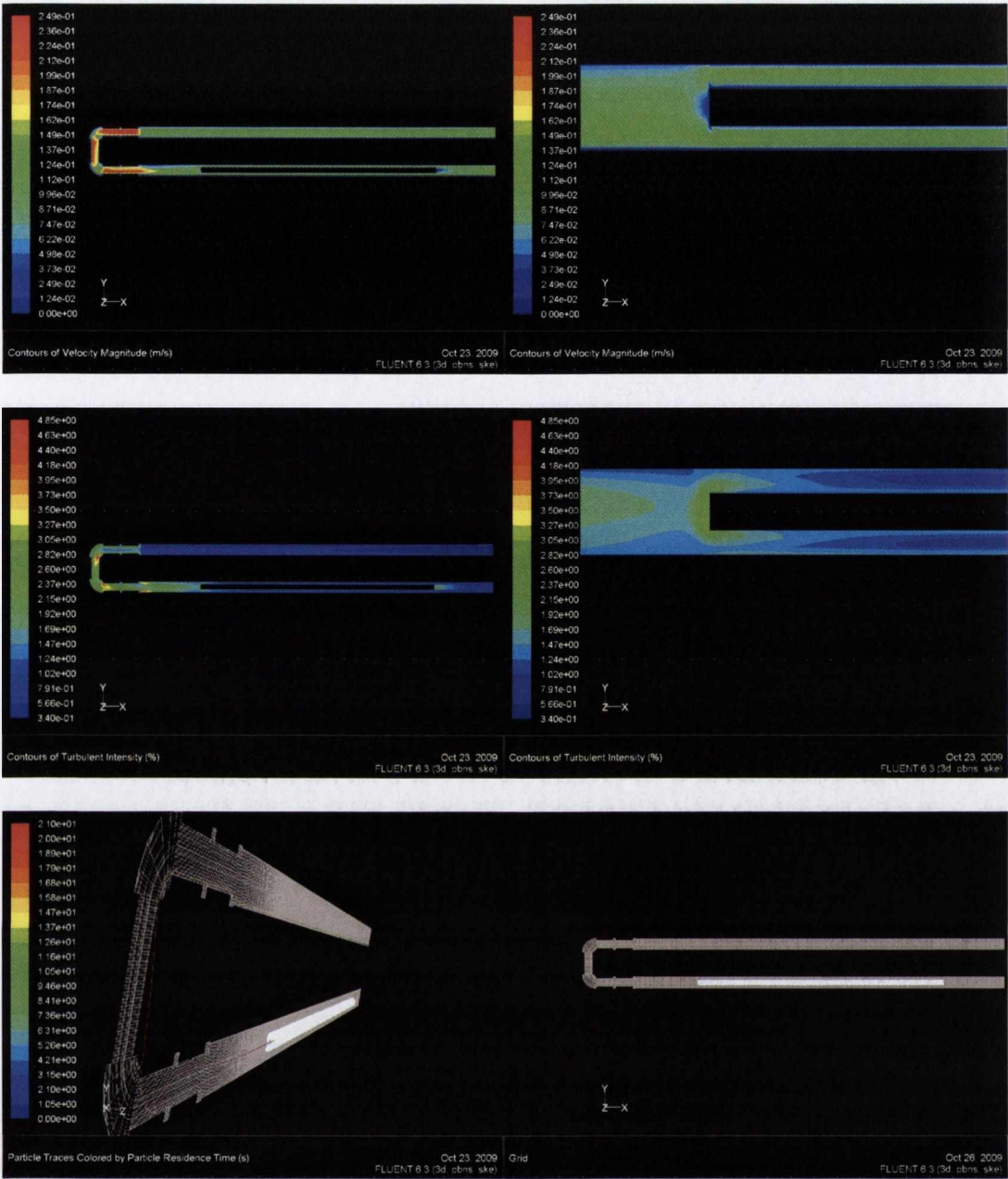
APPENDIX B



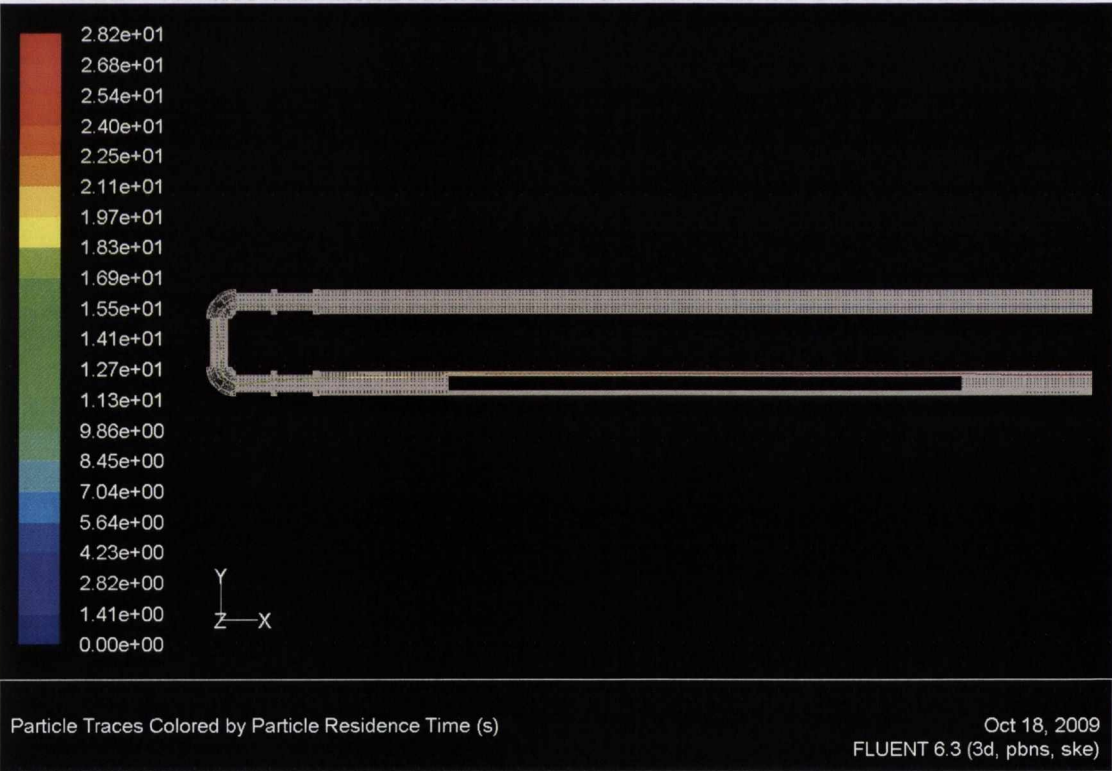
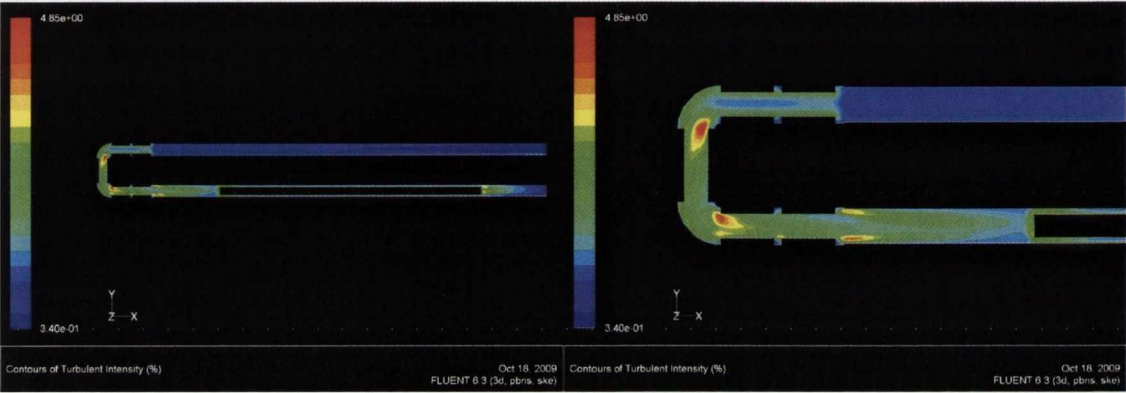
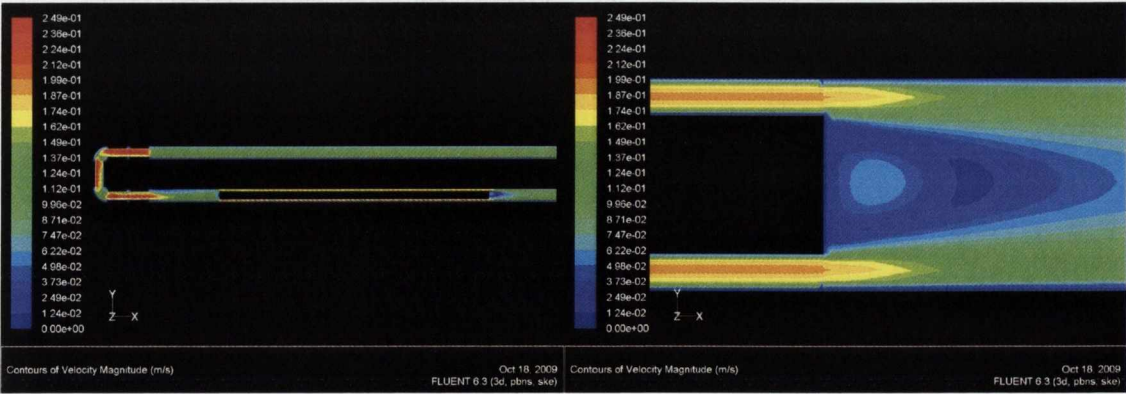
1. No insert



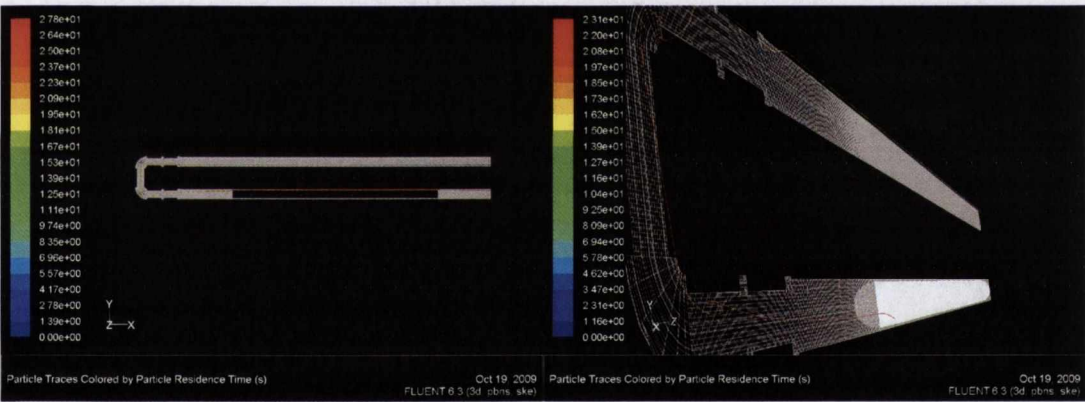
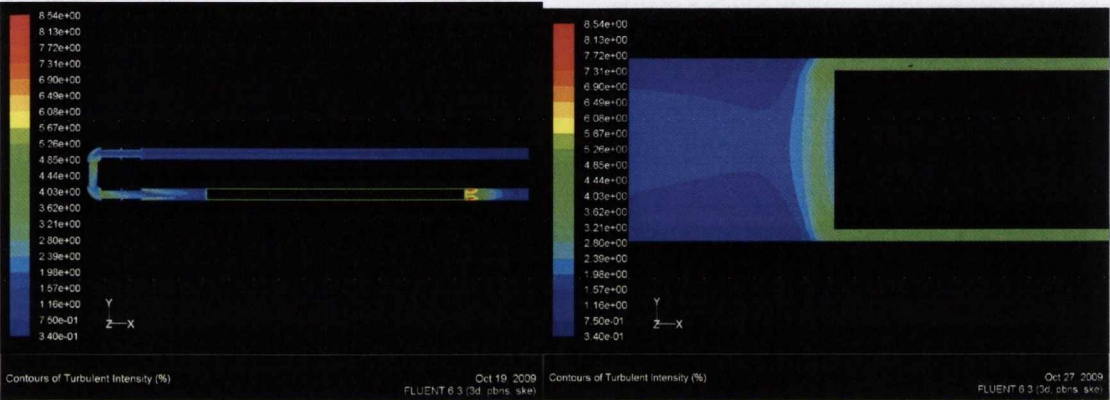
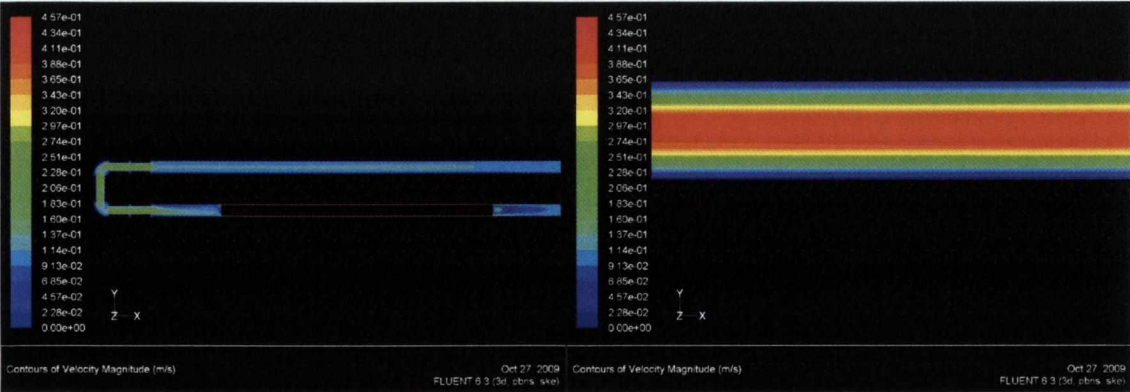
2. Cylinder, radius = 17.29 mm



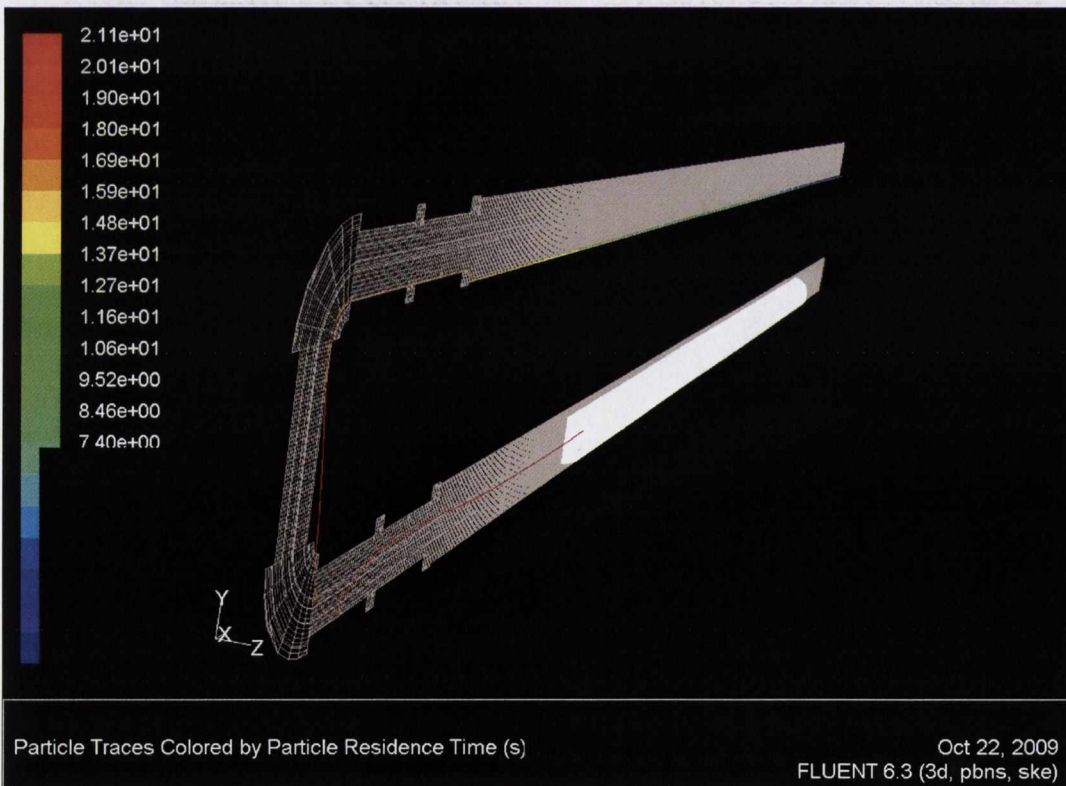
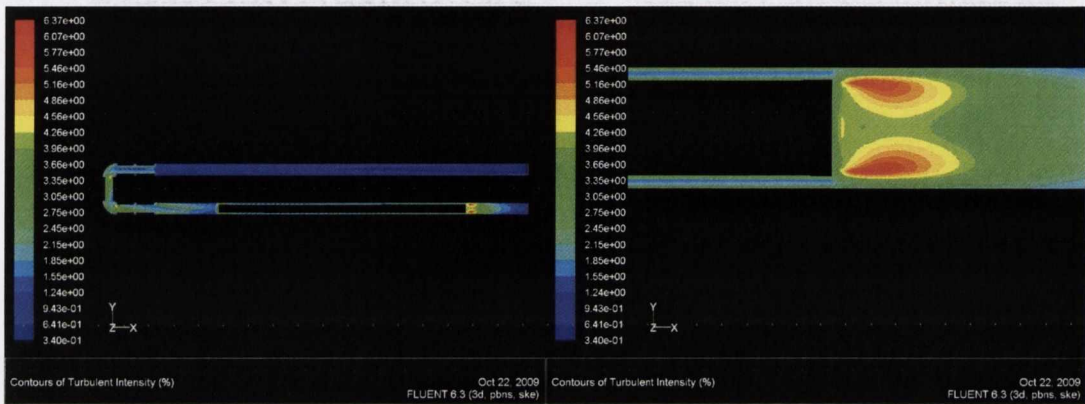
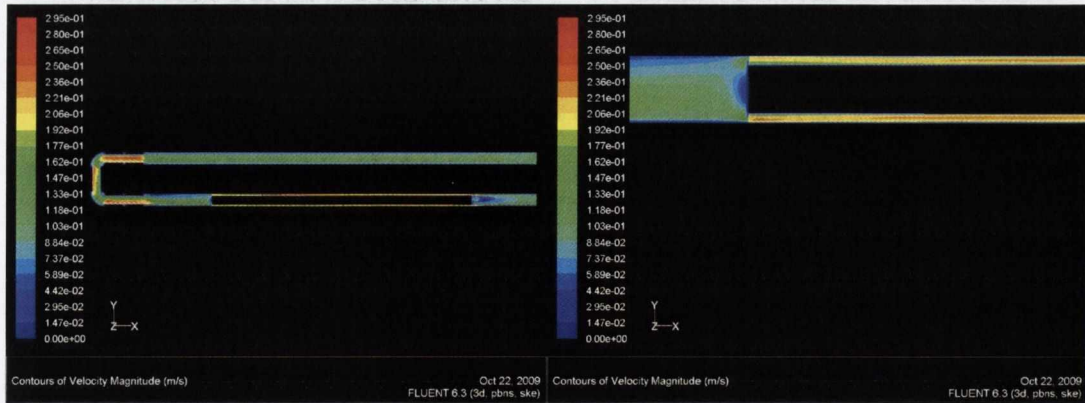
3. Cylinder, $r = 10\text{ mm}$



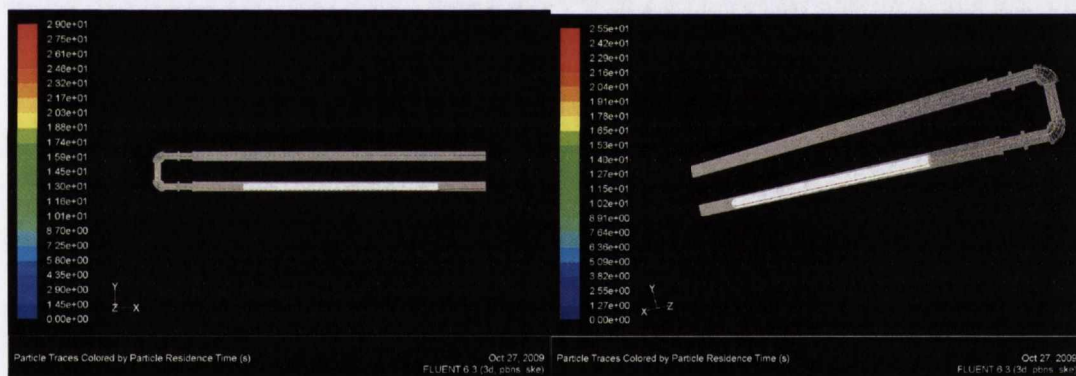
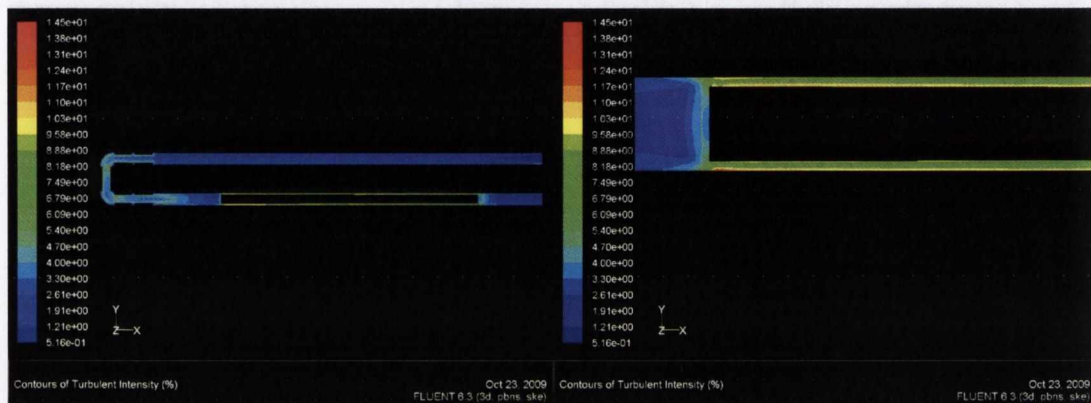
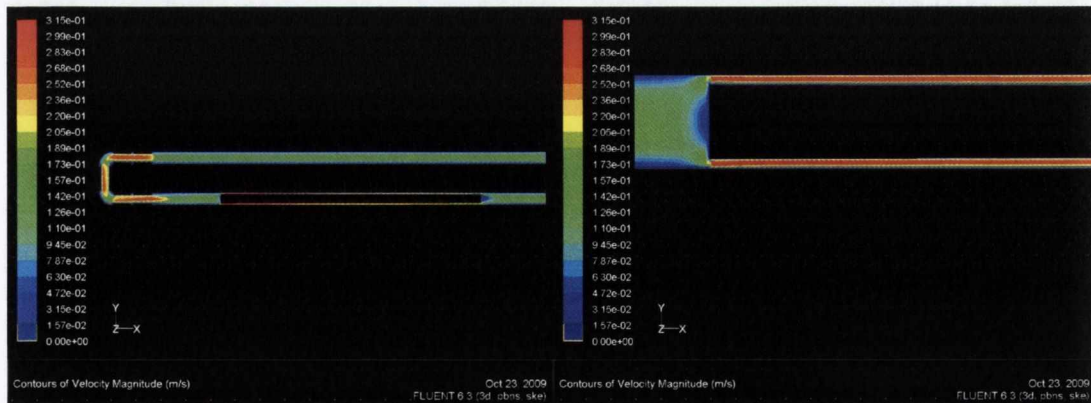
4. Cylinder, radius = 15 mm



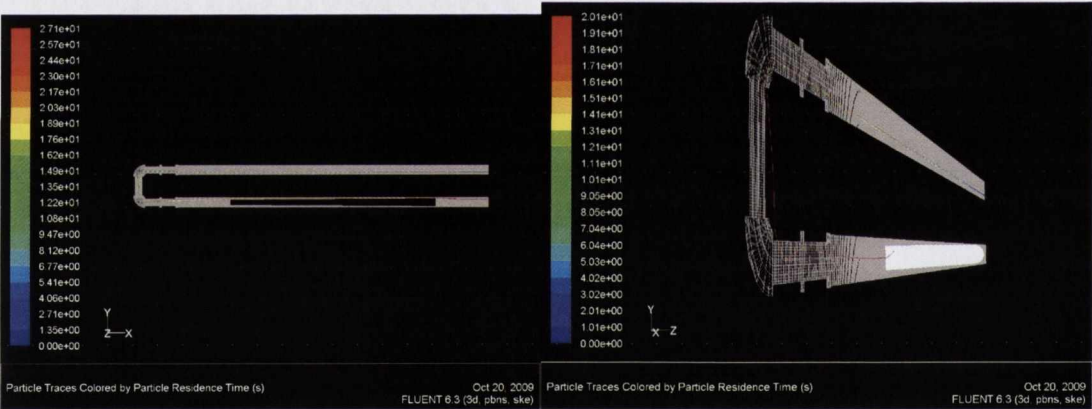
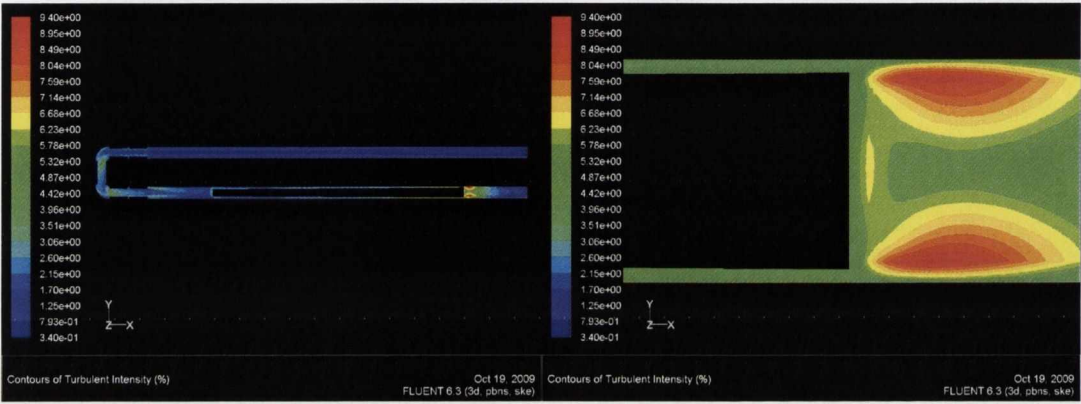
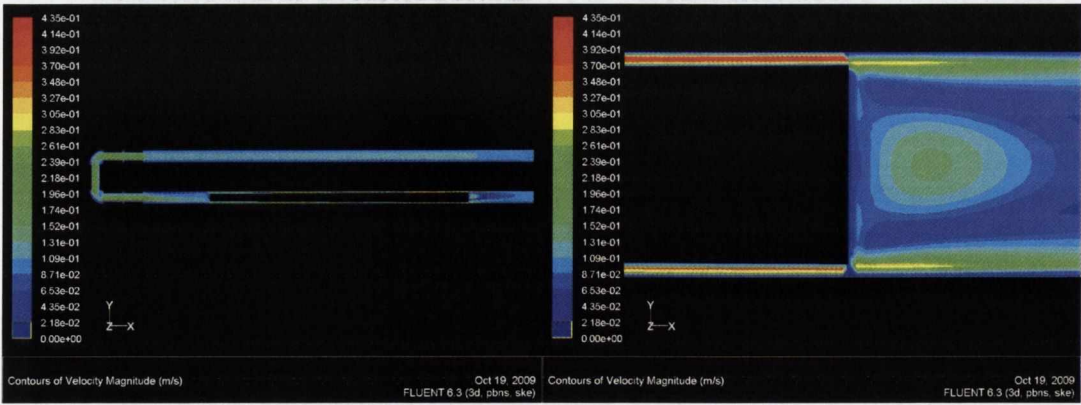
5. Cylinder, radius = 20 mm



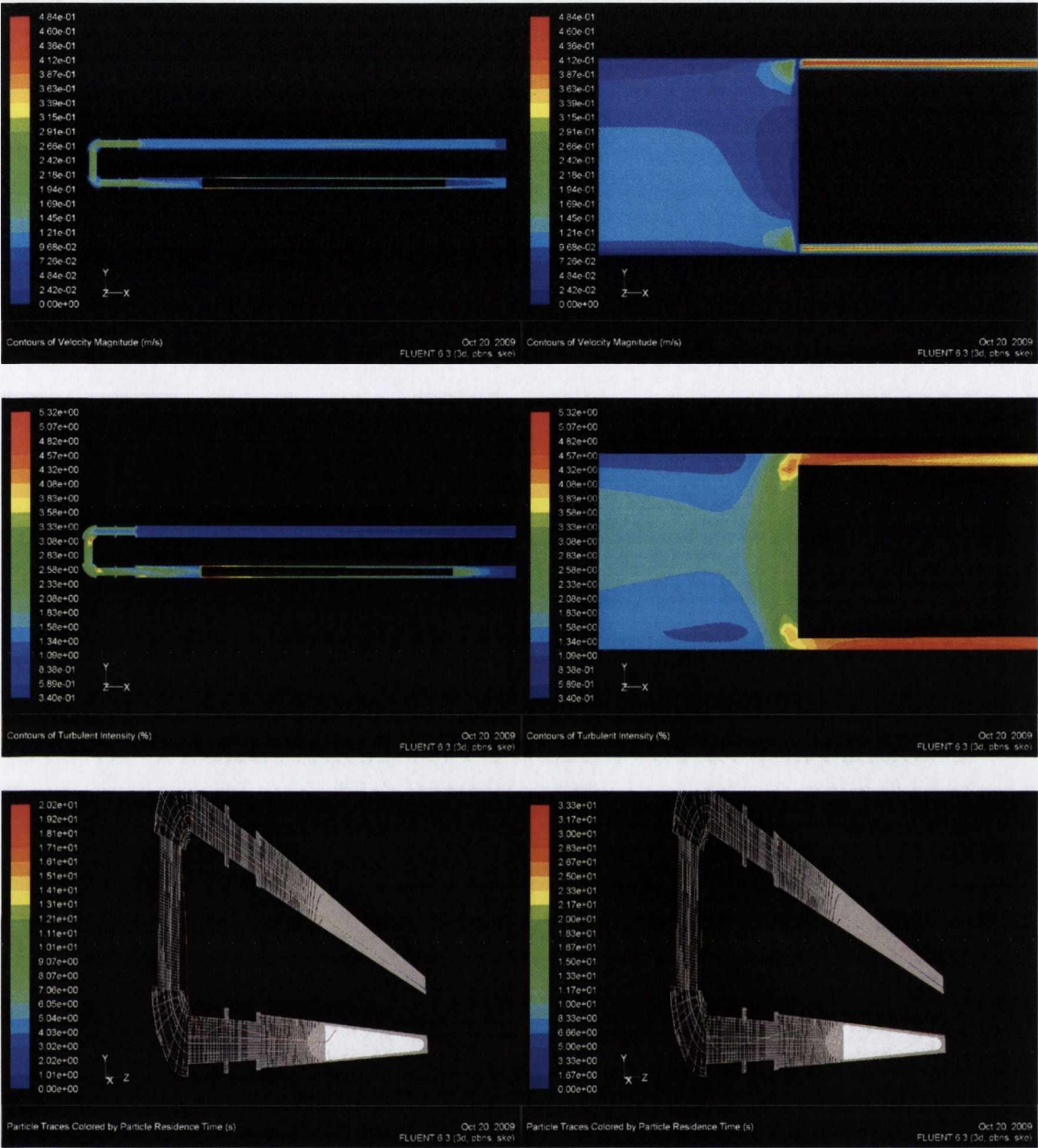
6. Frustum, $r_1 = 16.29$ mm, $r_2 = 18.29$ mm, radius increasing in direction of flow.



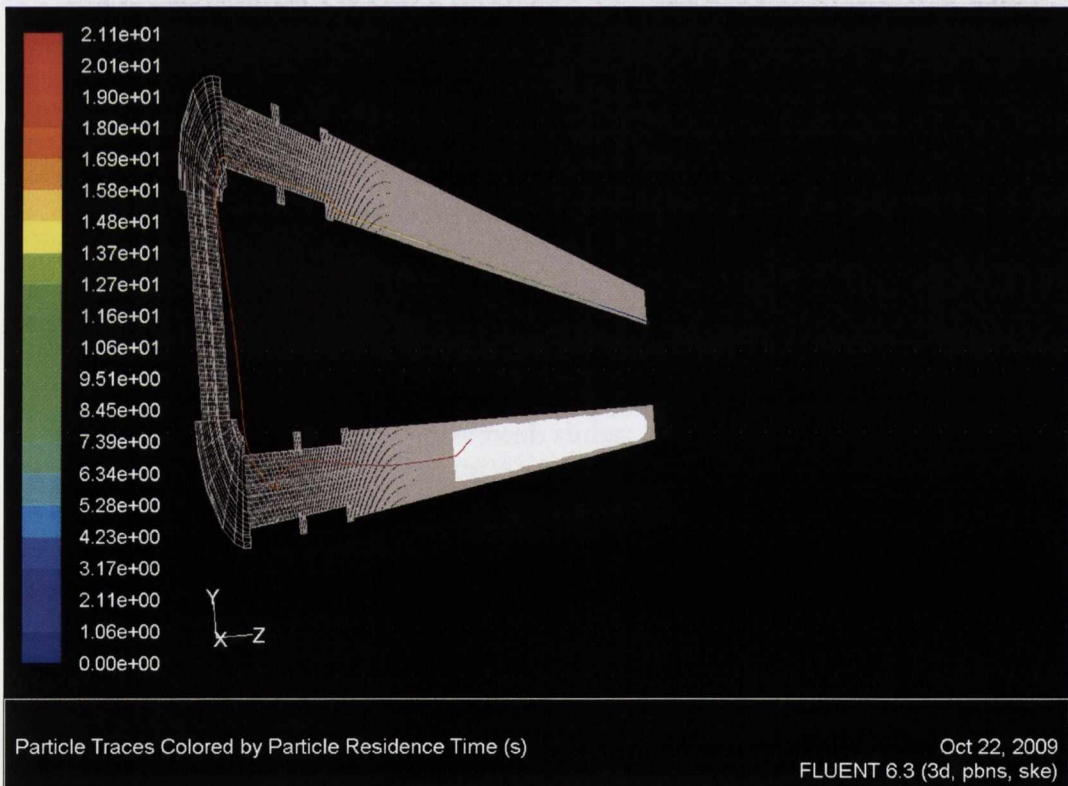
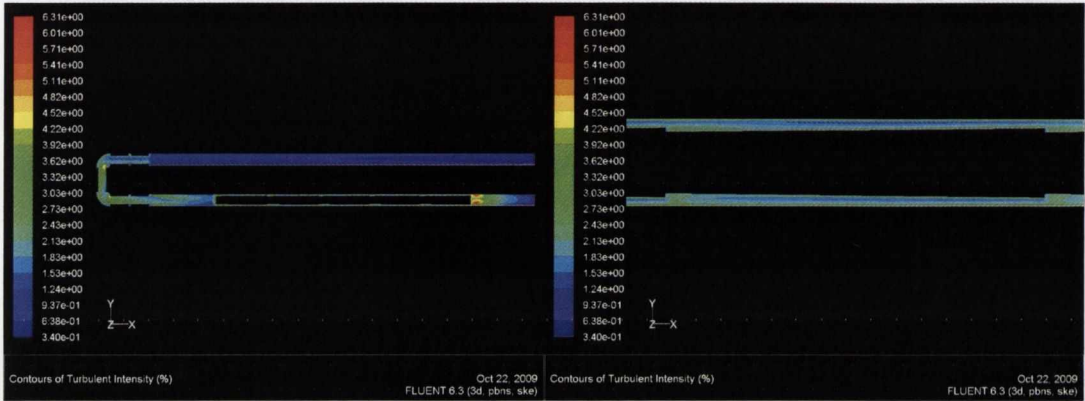
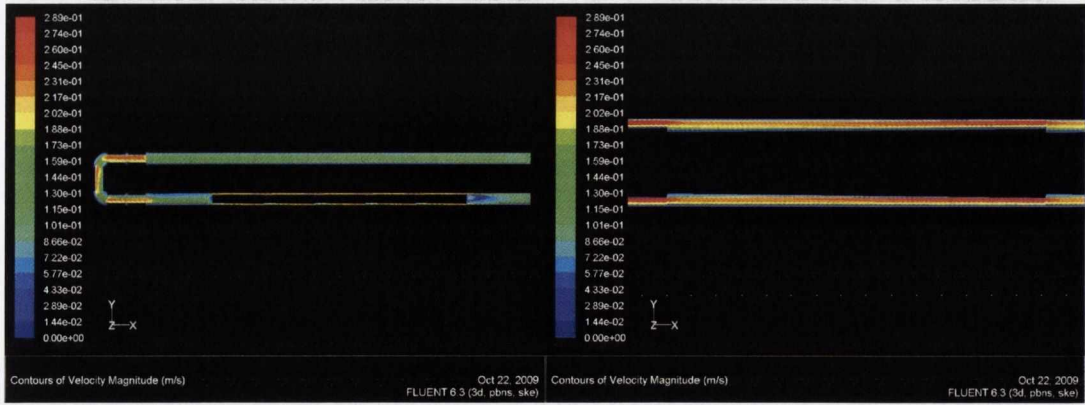
7. Frustum, $r_1 = 16.29$ mm, $r_2 = 18.29$ mm, radius decreasing in direction of flow.



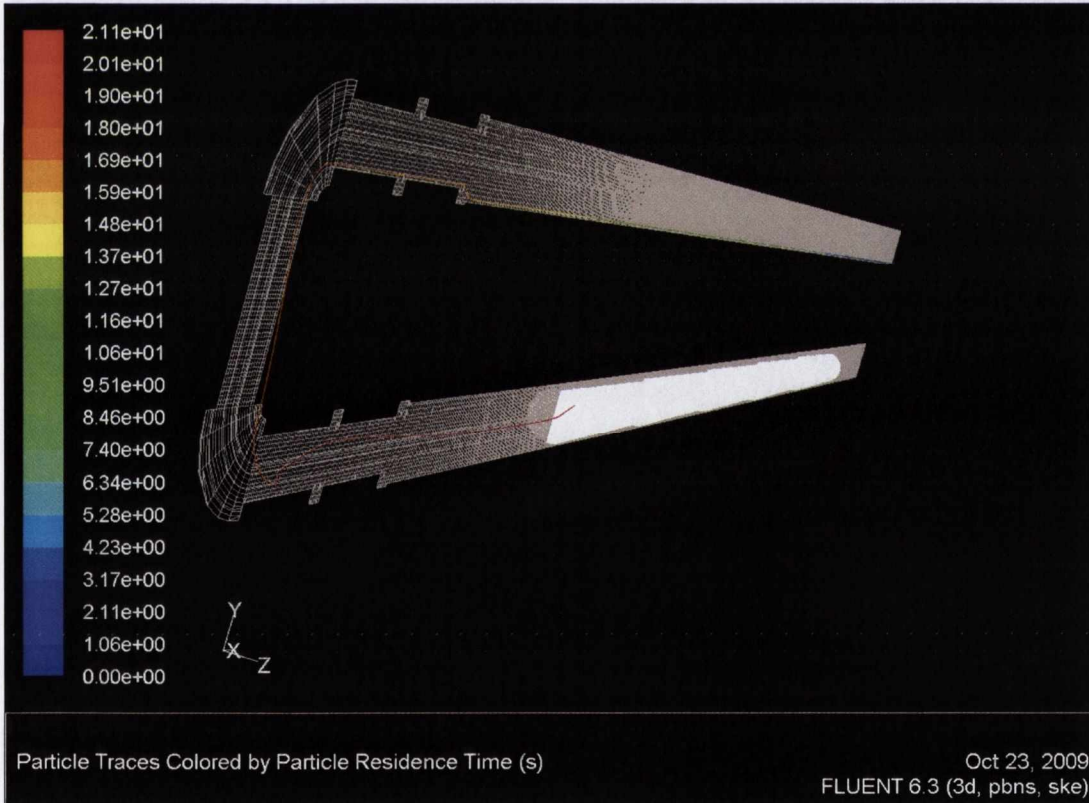
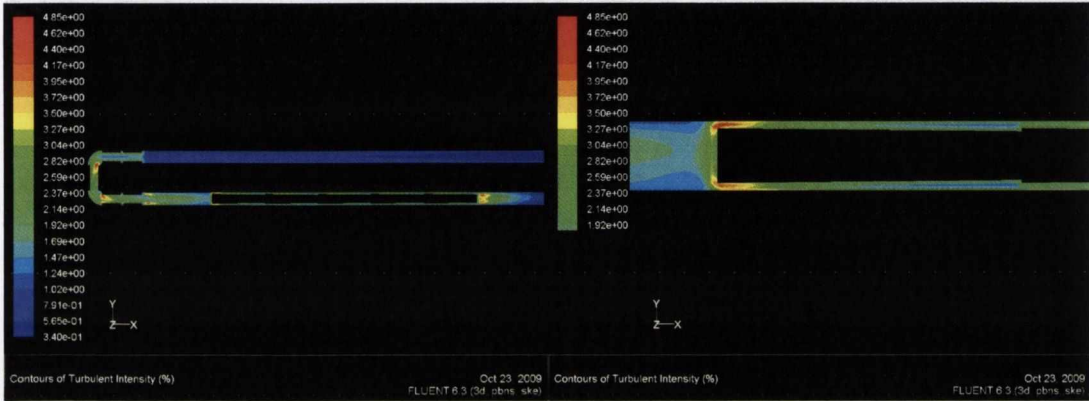
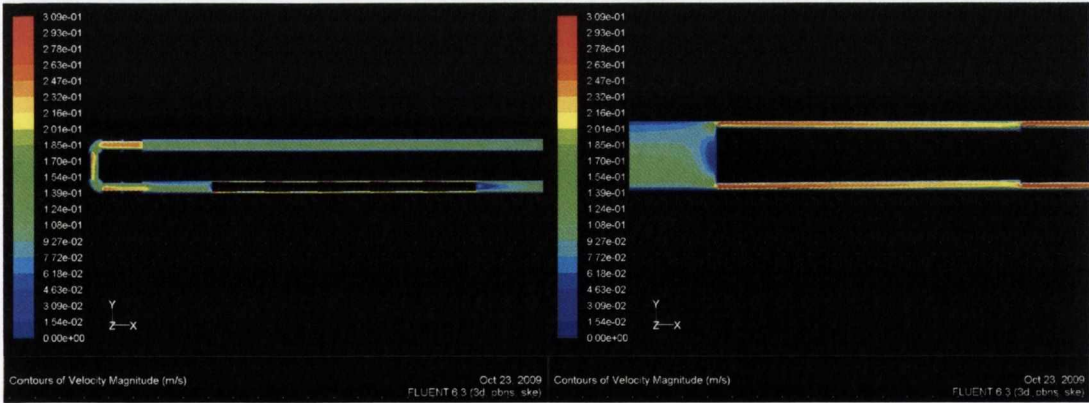
8. Frustum, $r_1 = 14.29$ mm, $r_2 = 20.29$ mm, radius increasing in direction of flow.



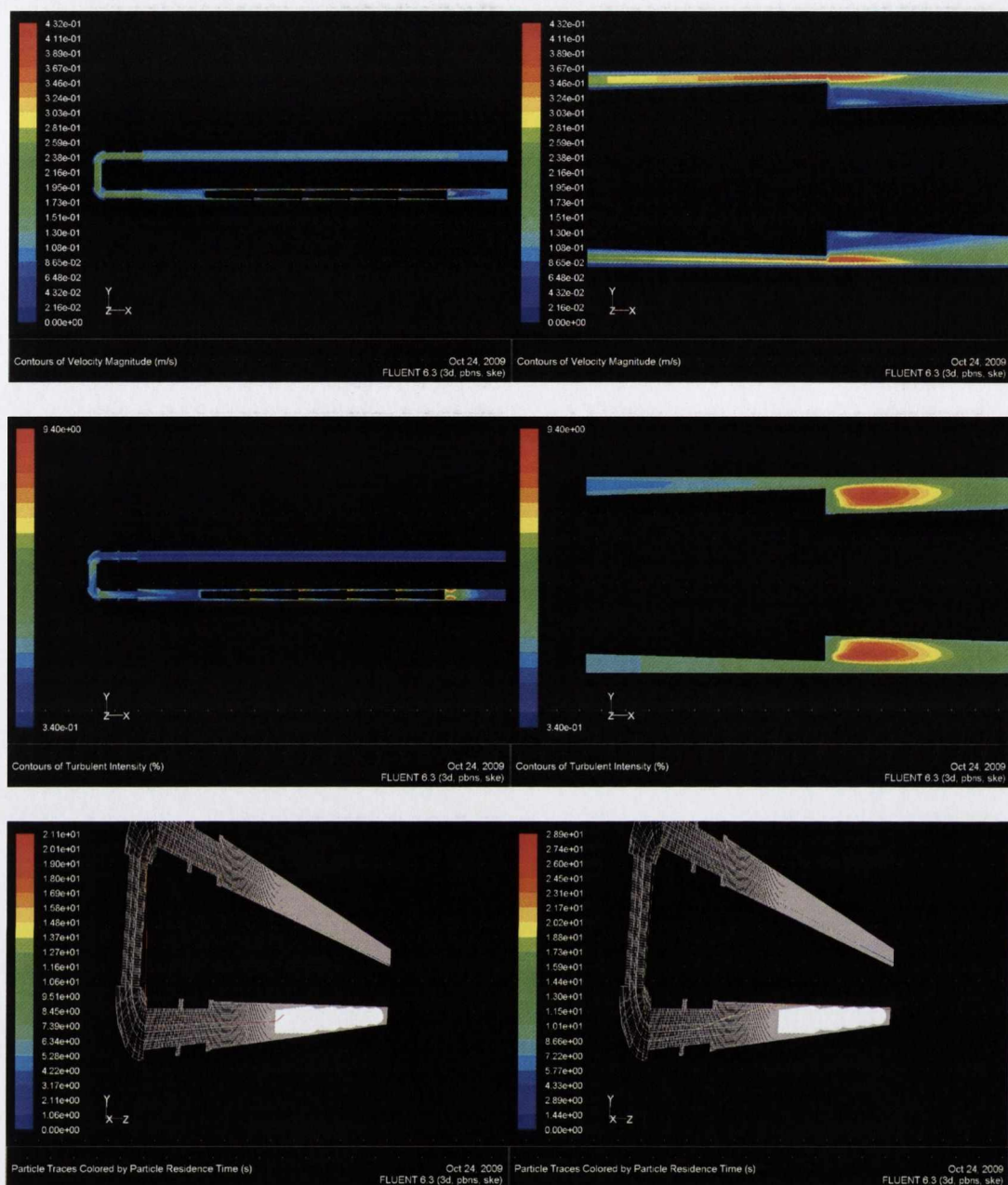
9. Frustum, $r_1 = 14.29$ mm, $r_2 = 20.29$ mm, radius decreasing in direction of flow



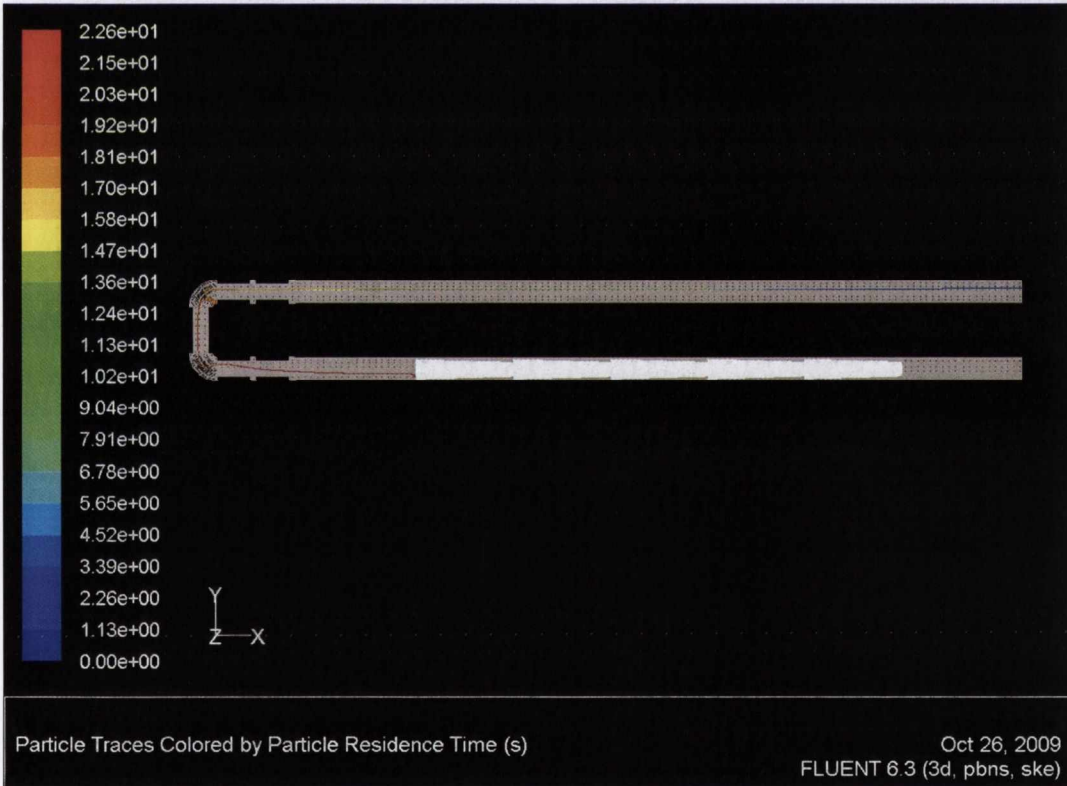
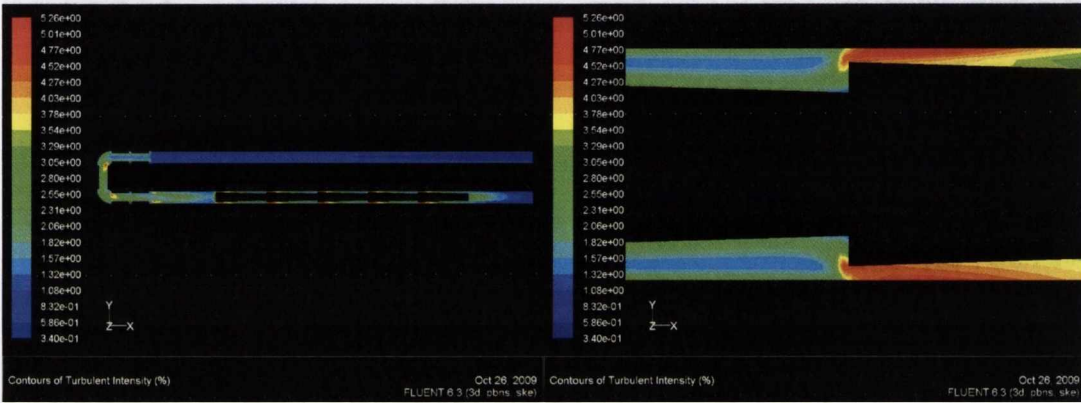
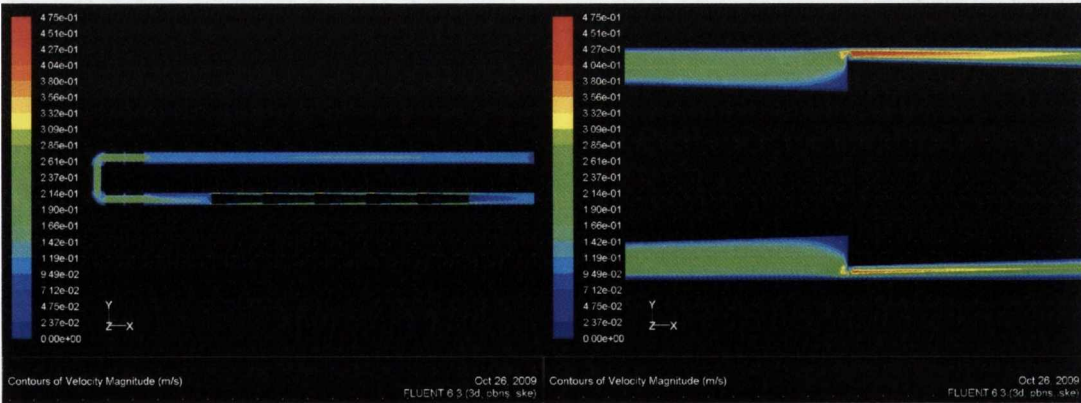
10. Arrangement of 5 frusta, $r_1 = 16.29$ mm, $r_2 = 18.29$ mm, radius of each frustum increasing in direction of flow.



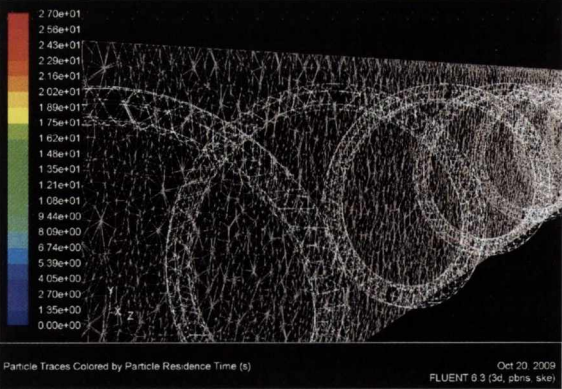
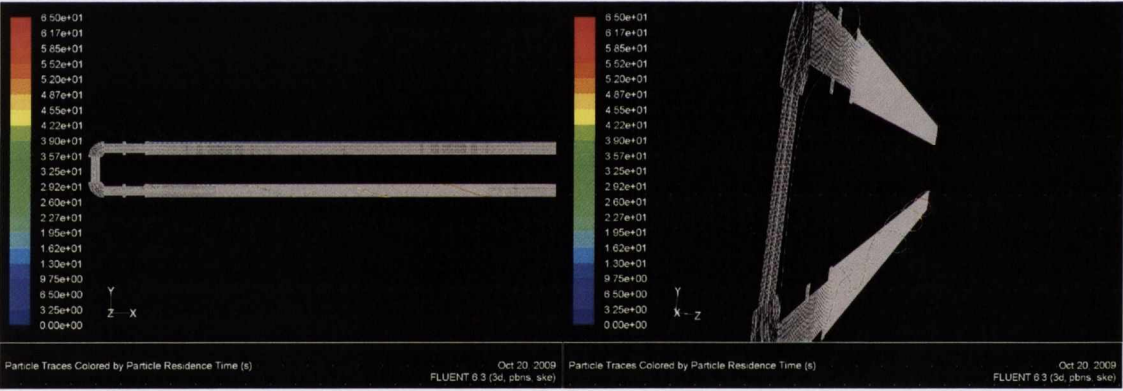
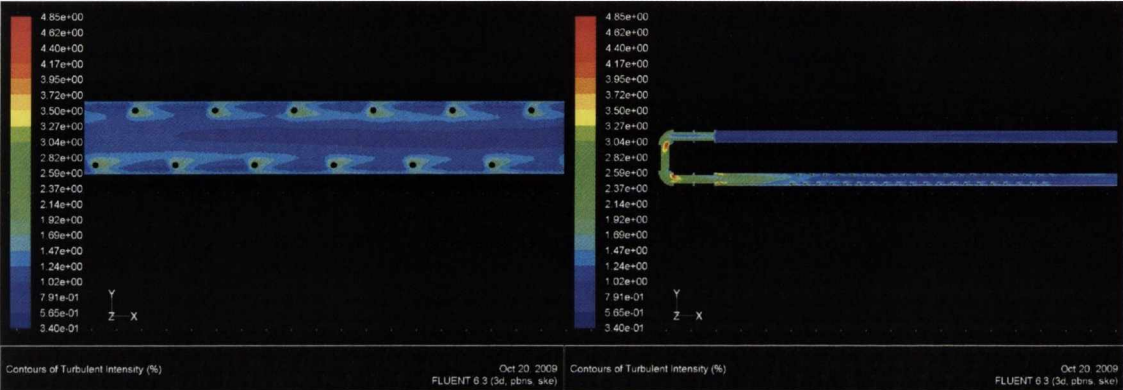
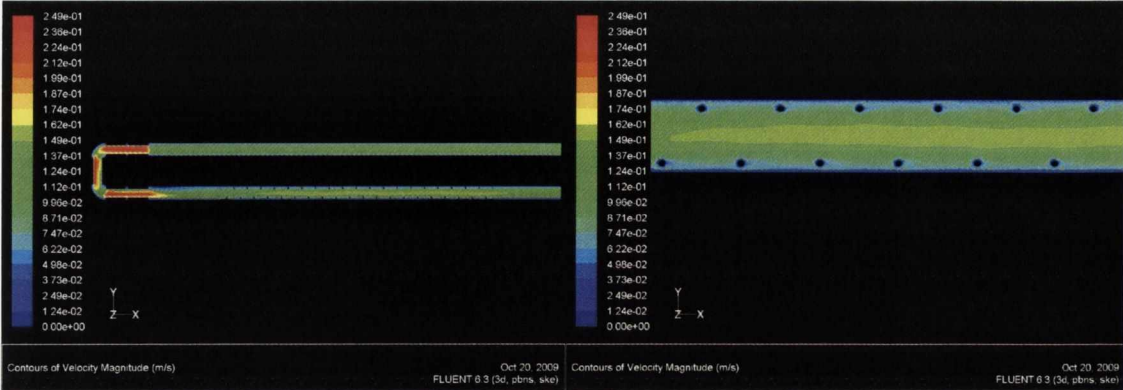
11. Arrangement of 5 frusta, $r_1 = 16.29$ mm, $r_2 = 18.29$ mm, radius of each frustum decreasing in direction of flow.



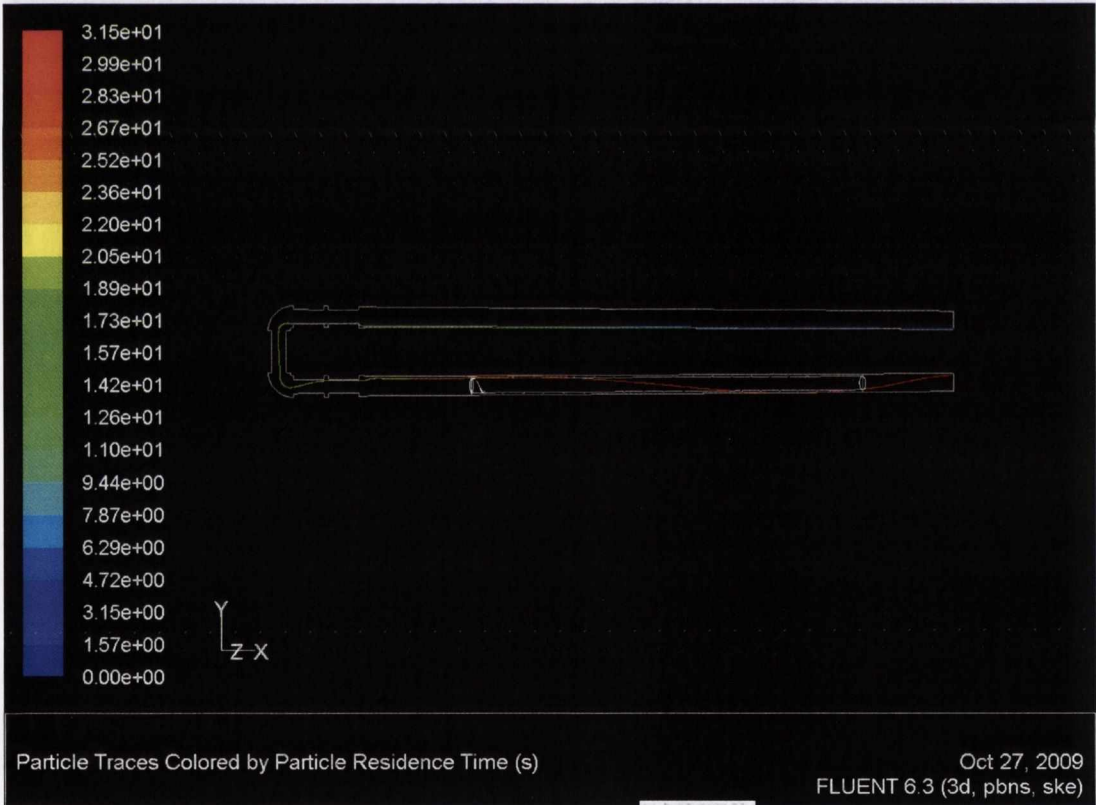
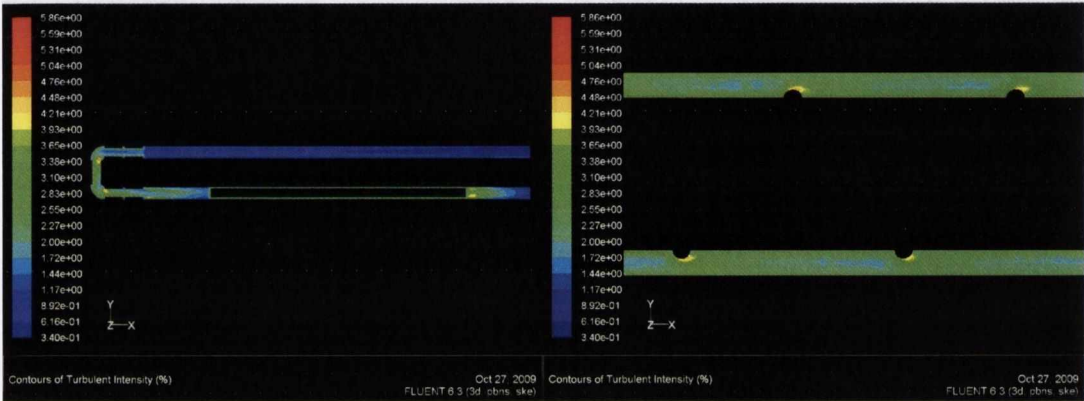
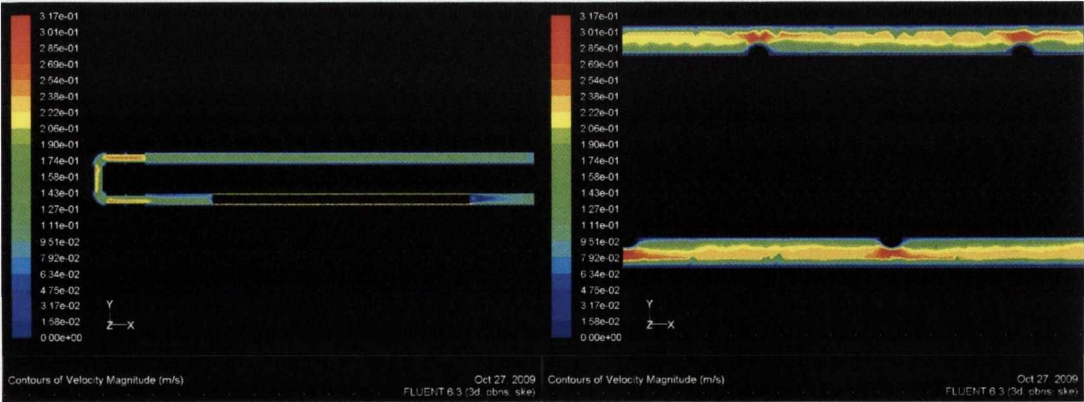
12. Arrangement of 5 frusta, $r_1 = 14.29$ mm, $r_2 = 20.29$ mm, radius of each frustum increasing in direction of flow.



13. Arrangement of 5 frusta, $r_1 = 14.29$ mm, $r_2 = 20.29$ mm, radius of each frustum decreasing in direction of flow.



14. Spring, tube radius = 2 mm, helix radius = 17.29 mm



15. Cylinder set inside helix, cylinder radius = 17.29 mm, tube radius = 2 mm, helix radius = 17.29 mm