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Solar Photocatalytic Disinfection of Plant Pathogen *Fusarium* Species

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Contents

I. Resumen

En general, en este trabajo se demuestra la acción desinfectante de la energía solar por si sola y combinada con la adición de TiO_2 y H_2O_2 sobre agua contaminada con esporas de *Fusarium*. Su eficacia no solamente se demuestra a escala de laboratorio, sino también en aplicaciones de volúmenes de varios litros en condiciones reales de desinfección, por lo que resulta viable el desarrollo completo de la desinfección de aguas contaminadas para su reúso en agricultura utilizando estas tecnologías.

La contaminación de aguas con microbios patógenos afecta no solamente al agua potable de una gran parte de la población mundial sino también a las aguas usadas o reutilizadas en la agricultura. Entre estos microorganismos, los hongos fitopatógenos típicamente constituyen un gran problema en los sistemas tradicionales de cultivos agrícolas y más en los sistemas de cultivo sin suelo (hidroponia). Entre estos hongos fitopatógenos, el género *Fusarium* es muy conocido por su resistencia a muchos tratamientos químicos. Su alta capacidad de adaptación a cualquier condición ambiental y su presencia en una gran variedad de suelos y aguas hacen que sea un patógeno muy importante en muchos cultivos.

Los tratamientos tradicionales con desinfectantes como el cloro o fungicidas orgánicos presentan problemas de resistencia de los hongos, fitotoxicidad o acumulación de residuos tóxicos. La alternativa que se estudia en este trabajo es el uso de la radiación solar para controlar los hongos fitopatógenos. Esta técnica pretende ser un método sostenible y eficaz contra una mayoría de patógenos sin generar residuos tóxicos.

En los capítulos de esta memoria se demuestra la capacidad desinfectante que tiene la radiación solar por sí misma y la fotocátalisis solar, haciendo uso del dióxido de titanio (TiO_2), para desinfectar agua contaminada con varias especies del genero *Fusarium*: *Fusarium equiseti, Fusarium oxysporum*, *Fusarium anthophilum, Fusarium verticillioides* y *Fusarium solani* (capítulo IV. 1). Este estudio se realizó a escala de laboratorio en botellas de vidrio de borosilicato de 250 mL utilizadas como reactores solares.

También de este modo se estudió el efecto del tratamiento solar combinado con TiO_2 sobre los diferentes tipos de esporas que produce el género *Fusarium*. En esta parte del trabajo se

encontró que las clamidosporas de *F. equiseti* son más resientes al tratamiento fotocatalítico que las micro y macroconidias, probablemente debido a la resistencia de la pared celular, ya que el semiconductor sobre todo actúa al ser adsorbido sobre la pared celular (capítulo IV. 2).

Aparte de demostrar la eficacia de dicho método sobre el género *Fusarium* este trabajo pretende desarrollar una aplicación a mayor escala sometiendo decenas de litros de agua contaminada al proceso de desinfección. Por ello se desarrolló un estudio de desinfección con reactores solares usando como modelo la bacteria *Escherichia coli* y catalizadores soportados (capítulo IV. 3). El objetivo de este trabajo es conocer la influencia de la radiación (dosis e intensidad), la concentración de catalizador, el régimen de flujo y el estrés osmótico en agua destilada sobre el proceso de desinfección.

El trabajo sobre la influencia de la energía solar recibida por los fitopatógenos durante el tratamiento y en las diferentes épocas del año mostró que la radiación solar es eficaz como desinfectante en días despejados de verano, pero la desinfección combinada con TiO_2 se puede usar durante prácticamente todo el año debido a su buena eficiencia en condiciones de radiación relativamente adversas (capítulo IV. 4).

Conociendo la influencia de dichos factores en la desinfección fotocatalítica con TiO_2 se logró realizar la desinfección de *F. solani* en colectores solares parabólicos compuestos, (CPCs) de 14 L por móulo y con agua real de pozo (sección V). El mayor obstáculo en llegar de la escala del laboratorio a módulos CPC con agua real resultó ser el cambio del régimen de flujo y de la matriz de agua. Debido al pH alto del agua de pozo (7.8) y el bajo régimen de flujo se produjo la sedimentación del catalizador, lo cual provocaba la pérdida de esporas de *F. solani* por sedimentación, sin ser inactivadas durante el proceso. En la sección V (resultados no publicados) se presentan los resultados a escala piloto y las medidas que se tomaron para resolver los problemas técnicos mencionados. Este logro permite demostrar que el nuevo método de desinfección se puede utilizar para el uso de desinfección de aguas de riego y probablemente también se puede utilizar para desinfectar agua recirculada en soluciones nutritivas en los sistemas agrícolas de cultivos sin suelo; si bien, hay que realizar más trabajo experimental en este campo.

Por otra parte, esta tesis investiga la capacidad desinfectante del agua oxigenada en muy bajas concentraciones (5, 10, 50 mg/L) en presencia de radiación solar (capítulo IV. 5). El agua oxigenada, junto con la radiación solar, presenta un efecto sinérgico dañino sobre los microorganismos en general y, como se demuestra en este trabajo, también sobre *F. solani*. En la literatura existen muy pocos trabajos sobre la inactivación sinérgica de H_2O_2 y radiación de solar (o UV-A simulada) sobre *E. coli* y el fago T-7, por lo que todavía no se ha aplicado este método para la desinfección de aguas de uso agrícola ni para inactivar ningún tipo de hongo. El trabajo realizado demuestra una gran eficacia del método con agua destilada y agua de pozo. Debido a su fácil aplicación, esta técnica permitirá la reducción significativa de la cantidad de agua oxigenada como desinfectante en agua y probablemente en un futuro también se podrá estudiar su aplicación en la desinfección de semillas.

Summary

This work has demonstrated the disinfecting action of solar energy, alone and in combination with added TiO_2 and H_2O_2 on water contaminated by *Fusarium*. Its effectiveness has not only been demonstrated in the laboratory, but also in volumes of several liters under real disinfection conditions, making complete development of the method from laboratory scale to application to the reality of water disinfection for irrigation or human consumption feasible.

Pollution of water by pathogenic microbes affects not only the drinking water of a large part of the world population, but also water used or reused in agriculture. Among these microorganisms, phytopathogenic fungi typically create a severe problem in traditional agricultural systems and more so in soilless cultures (hydroponics). Among these phytopathogenic fungi, the *Fusarium* genus is well known for its resistance to many chemical treatments.

Its high capacity for adapting to any environmental condition and its presence in a wide variety of soil and water make it a very important pathogen in many crops. Traditional treatment with disinfectants, such as chlorine or organic fungicides, meets with fungal resistance, phytotoxicity and accumulation of toxic waste. The alternative under study in this work is the use of solar radiation to limit phytopathogenic fungi. This technique is intended as an effective, sustainable method against most pathogens without generating toxic waste. In the chapter IV. 1 in this report, the disinfecting ability of solar radiation is demonstrated in a laboratory study, alone and in solar photocatalysis making use of titanium dioxide (TiO_2) in 250-mL borosilicate-glass solar reactors, to disinfect water polluted by various species of *Fusarium*: *Fusarium equiseti, Fusarium oxysporum*, *Fusarium anthophilum, Fusarium verticillioides* and *Fusarium solani*.

The solar treatment combined with TiO_2 of different types of *Fusarium* spores was also studied in this way. In this part of the work, it was found that *F. equiseti* chlamydospores are more resistant to photocatalytic treatment, probably because of the resistant cell wall, as the semiconductor acts mostly when it is adsorbed on the cell wall (chapter IV.2).

Apart from demonstrating the effectiveness of this method on the *Fusarium* genus, this work is intended to develop a larger-scale application using tens of liters of water in the disinfection process. Therefore, a disinfection study was also carried out in solar reactors using the bacteria *Escherichia coli* and supported catalysts. The purpose of this work is to find out the influence of radiation (dose and intensity), the catalyst concentration, the flow rate and osmotic stress in distilled water (chapter IV.3).

Work done on the influence of solar energy on phytopathogens during treatment and at different seasons of the year showed that solar radiation is most effective as a disinfectant on clear summer days, but combined disinfection with TiO_2 may be used almost all year around due to its good efficiency under relatively adverse radiation conditions (chapter IV.4).

Knowing the influence of these factors on photocatalytic disinfection with TiO_2, disinfection of *F. solani* in real well water in 14-L compound parabolic collector (CPC) modules was possible. The strongest obstacles for getting from the laboratory to the CPC modules with real water turned out to be the change in flow rate and the water matrix. Due to the high pH of the well water (7.8) and the low flow rate, the catalyst settled, causing loss of *F. solani* spores due to sedimentation without being inactivated by the process. Section V (unpublished results) shows the pilot-scale results and measures taken to solve the above mentioned technical problems. Its success demonstrates that the new method can be used for disinfection of irrigation water and probably also for disinfecting nutritive solutions in recirculating water in soilless cultivation systems, although more work should be done in this field (section V).

On the other hand, this thesis investigates the disinfecting ability of very low concentrations (5, 10, 50 mg/L) of peroxide in water in the presence of solar radiation (chapter IV.5). Hydrogen peroxide, along with solar radiation, has a synergistic effect harmful to microorganisms in general and, as demonstrated in this work, to *F. solani* also. There is very little in the literature on synergetic inactivation of *E. coli* or the phage T7 by H_2O_2 and solar radiation or simulated UV-A, and this method has still not been used for disinfecting water in agriculture, nor for inactivation of any fungus. The work done here

shows the excellent effectiveness of this method in distilled water and well water. Due to its easy application, this technique would significantly reduce the required amount of hydrogen peroxide as a disinfectant in water and probably in future its application for the disinfection of seeds could also be studied.

II. General Introduction

II.1. Traditional water disinfection methods

Drinking water: The most important issue in water disinfection is, of course, safe drinking water. According to the World Health Organization (WHO), UNICEF and the German Welthungerhilfe (DWHH), polluted drinking water and lack of sanitation is responsible for the death of approximately 4500 - 5000 children every day, and one billion people still lack access to safe drinking water [WHO/UNICEF, 2005; Welthungerhilfe, 2008].

The microbial contamination in different regions may have different anthropogenic and non-anthropogenic origins. If fecal wastewater from human settlements is not treated before being discharged into a river, it can cause contamination further downstream. Cattle-raising close to rivers can cause microbial contamination of river water which is later used for irrigation, washing and sometimes even drinking. **Table 1** shows an estimate of pathogens in lakes, rivers, streams and groundwater sources found in recent literature and summarized by the WHO Drinking-Water Guidelines [WHO, 2006].

Table 1 Examples of high detectable concentrations (in CFU per liter) of enteric pathogens and fecal indicators in different types of source waters from scientific literature [WHO, 2006].

Pathogen or Indicator group	**Lakes and reservoirs**	**Impacted rivers and streams**	**Wilderness rives and streams**	**Groundwater**
Campylobacter	20-500	90-2500	0-100	0-10
Salmonella	-	3-58000	1-4	-
E. coli (generic)	10 000-1 000 000	30 000 -1 000 000	6 000-30 000	0-1 000
Viruses	1-10	30-60	0-3	0-2
Cryptosporidium	4-290	2-480	2-240	0-1
Giardia	2-30	1-470	1-2	0-1

Except for groundwater and the low figures in wilderness rivers and streams, all detected concentrations are infectious concentrations of pathogens highly significant for health. Therefore water disinfection is essential for the consumption of safe water.

Drinking water disinfection is primarily defined by the destruction of microorganisms causing epidemic diseases, especially cholera and typhoid fever. The mechanism involved is most commonly explained as the destruction of the organism protein structure and inhibition of the enzymatic activities [Lawrence and Block, 1983]. This definition leads to the general resistance order to widely-used high-level disinfectants such as ozone and chlorine compounds (**Table 2**):

Table 2 Classification of microorganisms according to their sensitivity to disinfectants [after Rutala and Weber, 2004].

High → **Middle** **Resistance**		
Prions (Creutzfeldt-Jakob Disease Agent)	Coccidia (*Cryptosporidium*) Bacterial spores (*Bacillus*)	Mycobacteria (*M. tuberculosis*)
Middle → **Low** **Resistance**		
Non-enveloped viruses (poliovirus), Fungi (*Aspergillus*)	Gram-negative bacteria (*Pseudomonas*)	Gram-positive bacteria (*Staphylococcus, Enterococcus*) enveloped viruses (HIV)

This resistance to common water disinfectants is partly determined by the size and complexity of the microorganism, but more decisively by its cell wall permeability to the specific disinfectant [Rutala and Weber, 2004]. **Table 3** shows the resistance of microorganisms to chlorine along with their significance for water supply, and their risk to human health [WHO, 2006].

It may be observed that most bacteria, except the non-tuberculous mycobacteria, are relatively easy targets for chlorine disinfection. Nevertheless, they must be taken seriously because they are highly infectious and can multiply in water supply systems. All the viruses mentioned can easily cause infections highly significant for health. Furthermore, they are moderately resistant to chlorine disinfection and persistent in water supply systems.

Table 3 Waterborne pathogens and their significance in water supplies [WHO, 2006].

Pathogen	Health significance	Persistence in water supplies	Chlorine resistance	Relative infectivity	Important animal source
Bacteria					
Burkholderia pseudomallei	Low	May multiply	Low	Low	No
Campylobacter jeununi, C. coli	High	Moderate	Low	Moderate	Yes
Escherichia coli-Pathogenic	High	Moderate	Low	Low	Yes
E. coli-Enterohaemorrhagic	High	Moderate	Low	High	Yes
Legionella spp.	High	Multiply	Low	Moderate	No
Non-tuberculous mycobacteria	Low	Multiply	High	Low	No
Pseudomonas aeruginosa	Moderate	May multiply	Moderate	Low	No
Salmonella typhi	High	Moderate	Low	Low	No
Other *salmonellae*	High	May multiply	Low	Low	No
Shigella spp.	High	Short	Low	Moderate	No
Vibrio cholerae	High	Short	Low	Low	No
Yersinia enterocolitica	High	Long	Low	Low	Yes
Viruses					
Adenoviruses	High	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A virus	High	Long	Moderate	High	No
Hepatitis E virus	High	Long	Moderate	High	Potentially
Noroviruses and saproviruses	High	Long	Moderate	High	Potentially
Rotaviruses	High	Long	Moderate	High	No
Protozoa					
Acanthamoeba spp.	High	Long	High	High	No
Cryptosporidium parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolyticaa	High	Moderate	High	High	No
Giardia intestinalis	High	Moderate	High	High	Yes
Naegleria fowleri	High	May multiply	High	High	No
Toxoplasma gondii	High	Long	High	High	Yes

The most difficult targets for chlorine disinfection are the protozoa, which are infectious at very low concentrations and are moderately to highly persistent in water systems. Therefore protozoa in potential drinking water systems are very alarming and have to be considered in the choice of treatment.

The WHO Drinking-Water Guidelines evaluates the use of the following drinking water treatment techniques [WHO, 2006]:

Pre-treatment:

- Coagulation/flocculation/sedimentation,
- Filtration

Disinfection:

- UV-C-Disinfection,
- Ozonation,
- Chlorine,
- Monochloramine,
- Chlorinedioxid

.

Coagulation, flocculation and sedimentation remove a maximum of 90% of bacteria, 70% of viruses and 90% of protozoa [WHO, 2006]. Filtration for drinking-water treatment includes granular, slow sand, precoat and membrane filtration. Only with proper design and adequate operation, can filtration act as a consistent and effective barrier for microbial pathogens (approx. 99% bacteria removal) [WHO, 2006]. Depending on the water source, the remaining bacteria might still be able to cause disease, which makes filtration a good pretreatment, but not a completely safe disinfection technique. For highly resistant microorganisms like *Cryptosporidium* oocysts, filtration in combination with chlorine is recommended [WHO, 2006].

UV-C-Disinfection and ozonation always have associated installation, electricity and maintenance costs. But both techniques are very effective in killing bacteria and reasonably effective in inactivating viruses (depending on type) and many protozoa, including *Giardia* and *Cryptosporidium*. 99% of bacteria can be removed with 0.02 mg of ozone per min and liter at 5 °C and pH 6 – 7. For the disinfection of *Cryptosporidium,* the highest ozone

concentration is needed: 40 mg per min and liter at 1 °C. Despite its highly efficient inactivation of all microorganisms present, ozonation can also produce disinfection by-products (DBPs) (**Table 4**), depending on source-water quality.

Application of UV-C-lamps ($190<\lambda<290$nm) may also be limited depending on source-water turbidity [Masschelein, 2002]. But for clear water, average bacteria inactivation is 99% at 7 mJ/cm^2 and for *Cryptosporidium* at 5 mJ/cm^2 [WHO, 2006]. The total number of microorganisms reduced by UV-C is very similar for protozoa and bacteria, because of their similar susceptibility to UV-C damage. That is, the cell wall that protects them from chemical disinfectants is no protection against the UV-C radiation. Another advantage is that UV-C disinfection makes the storage and transport of reagents is unnecessary.

Chlorine is a very effective disinfectant for most microorganisms. 99% of bacteria can be killed with 0.08 mg per min and liter at 1 – 2 °C and neutral pH. For disinfection of 99% of viruses, 12 mg per min and liter at 0 – 5 °C and neutral pH is sufficient, but for the inactivation of 99% of *Giardia*, 230 mg per min and liter are necessary at 0.5 °C and neutral pH, and *Cryptosporidium* cannot be safely inactivated with chlorine at all [WHO, 2006].

The protozoa *Cryptosporidium, Giardia* and *Acanthamoeba,* which are very highly resistant to chlorine, present a high risk of infection and are extremely persistent in water supply systems (**Table 3**). Such significant resistance makes it clear that alternatives to chlorine as a general disinfectant must be found. These protozoa, as well as *C. jejuni, C. coli, Y. enterocolitica, P. aeruginosa*, have been successfully inactivated by solar or solar photocatalytic disinfection, as described further below.

Another alarming disadvantage of chlorine, is the appearance of its by-products, organohalides, especially trihalomethanes (THMs), in chlorinated drinking water. Organohalides were first found in the 1970s, and later fully characterized (**Table 4**) [Bellar *et al.,* 1974; Latifoglu, 2003]. These findings have led to severe criticism of its use in drinking water and even in irrigation water.

Table 4 Disinfection by-products present in disinfected waters treated with chlorine and ozone (IPCS, 2000)

Disinfectant	**Significant organohalogen products**	**Significant inorganic products**	**Significant non-halogenated products**
Chlorine/ hypochlorous acid	THMs, haloacetic acids, haloacetonitriles, chloral hydrate, chloropicrin, chlorophenols, halofuranones, N-chloramines, bromhydrins	chlorate (mostly from hypochlorite use)	aldehydes, cyanoalkanoic acids, alkanoic acids, benzene, carboxylic acids
Chlorine dioxide chloramine	haloacetonitriles, cyanogen chloride, organic chloramines, chloramino acids, chloral hydrate, haloketones	nitrate, nitrite, chlorate, hydrazine	aldehydes, ketones
Ozone	bromoform, monobromoacetic acid, dibromoacetic acid, dibromoacetone cyanogen bromide	chlorate, iodate, bromate, hydrogen peroxide, hypobromeous acid, epoxides, ozonates	aldehydes, ketoacids, ketones, carboxylic acids

Research seeking alternative methods of drinking water disinfection is necessary to solve the abovementioned limitations and issues. Any such solution will have to take into account many factors: i) cost, ii) power consumption, iii) sustainability, iv) absence of negative effects on health, and taste.

Irrigation water: In addition to the well-known task of drinking water disinfection, the second most critical issue is the disinfection of water for agriculture. According to the Food and Agriculture Organization of the United Nations (FAO), agriculture consumes 70% of fresh water used worldwide. In developing countries, this increases to over 95% of the available fresh water. The average water used for crops is around 1000 - 3000 m^3 per ton of cereal harvested, or in other words, 1-3 tons of water are used to grow 1 kg of cereal. Bearing in mind that the daily drinking-water requirement per person is only 2 - 4 liters, it is often forgotten that it still takes 2000 to 5000 liters of water to produce a person's daily food requirement [FAO, 2008].

80% of land cultivated worldwide is today still exclusively rainfed, and supplies over 60% of the world's food. Irrigation could triple or quadruple this production. However, the FAO does predict a sharp increase in irrigation replacing rainfed agriculture [FAO, 2008].

Stored rainwater or surface water used for irrigation accumulates phytopathogens as phytopathogenic bacteria and fungi can be found almost everywhere. In the Spanish provinces of Granada and Almería, for example, fungal pathogens like *Pythium, Phytophthora* and *Olpidium* have been reported in rivers, probably brought there by spores suspended in dust in the troposphere [Gomez, 1993 and 2003]. Phytopathogens like *Fusarium oxysporum, Fusarium solani, Fusarium moniliforme, Fusarium equiseti,* and *Phytophthora citrophtora* are transported long distances and have even been found in seawater along the coasts of the provinces of Granada and Almería in Southern Spain [Núñez *et al.,* 2006]. The plant and human pathogen *F. solani* has been reported as found almost everywhere, and in 2001, even in hospital water distribution systems [Anaissie *et al.,* 2001].

Phytopathogens may be fought with organic disinfectants, also referred to as pesticides, which can be employed for disinfection of irrigation water, but this involves many negative aspects (see below). Chlorine is used extensively, but has the drawback of the abovementioned disinfection by-products and resistance (see **Table 3** and **4**). Furthermore, depending on how crops are cultivated, phytotoxicity has also been reported [Jarvis, 1998].

Non-chemical disinfection methods are rare in agriculture, but some work on UV-C disinfection of pathogens, such as *Phytium* and *Phytophtora*, is repoted in the literature [Runia, 2001 and 1994].

Hydrogen Peroxide is also commonly used as a disinfectant in agriculture [Chikthimmah *et al.,* 2005], but it quickly becomes phytotoxic, especially in hydroponic cultures, at doses as low as 50 mg/L [Coosemans, 1995]. Thus to improve its efficacy, H_2O_2 has been combined with germicidical UV-C radiation or ozone [Sharpless *et al.,* 2003]. Both techniques are in use, but energy costs are very high.

Recent research on solar and solar photocatalytic disinfection attempts to combine sustainability with low cost leading to an efficient disinfection method, not only for drinking water, but also for irrigation.

II. 2. Pesticides as disinfectants in agriculture

Since production of all kinds of chemicals began to increase in what is often referred to as the "Chemical Age" in the 1950s, phytopathogens have often been fought with organic chemicals called "pesticides", which includes any chemical used to inactivate or control pests. In agriculture, this refers to herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), and rodenticides (rats and mice).

Benefits of these agricultural techniques, well known under such terms as the "Green revolution", come from pesticides, especially in regions where food production relied on monocultures. Unfortunately, pesticides have also other not so desirable effects in the consequent disruption of predator-prey relationships and loss of biodiversity, which pose serious threats to the long-term survival of major ecosystems. Of course pesticides can also have significant consequences for human health.

Perhaps the most glaring regional example of pesticide pollution harming human health is in the Aral Sea, where pesticides have been linked to cancer, pulmonary and haematological morbidity, congenital deformities, etc., and even immune system deficiencies [Ongley, 1996].

Water contamination caused by the biocide pentachlorophenol, which is used worldwide, and only banned in Europe in the last few years [WHO, 1998], is well known. In 1995, Wang and Lin reported that tetrachlorohydroquinone, a toxic metabolite of pentachlorophenol, produces significant dose-dependent damage to DNA [Wang and Lin, 1995]. The extent of its bioavailability was so threatening at one time that it was estimated that 30-40 tons of pentachlorophenol were being transported by the Rhine per year [WHO, 1987].

Another alarming WHO study reported significant levels of highly toxic polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in human breast milk [WHO 1989, 1996]. Besides the shockingly high accumulation levels in plants and humans, these substances are often not biodegradable and therefore imply indefinite long-term effects on the health of humans and

of whole ecosystems [WHO, 1987; Sadler *et al.*, 2005]. These substances are some of the list of persistent bioaccumulative and toxic (PBT) pollutants, which environmental organizations, such as the US Environmental Protection Agency (EPA), are fighting against [EPA, 2000]. Many of these PBTs, like aldrin, dieldrin, chlordane, DDT, mirex, and toxaphene are now better monitorized and controlled, especially since the US-Environmental Protection Agency (EPA) launched its PBT program in November 1998. But the fight against PBTs goes on. Not only is a reduction in the use of PBTs an issue, but also keeping new PBTs from entering the marketplace [EPA, 2000].

Specific control of phytopathogenic fungi by fungicides, has had very limited success. This is due to their easily acquired resistance to systemic inhibitors [Kunz *et al*., 1997; Metcalfe *et al*., 2000]. A recent publication by Metcalfe *et al*. compares three common fungicides, flutriafol (very mobile within leaves), fluquinconazole (less mobile), and prochloraz (almost immobile). All are sterol demethylation inhibitors (DMIs). DMIs inhibit cytochrome P450 14α-demethylase [Walsh and Dixon, 1996]. This enzyme converts lanosterol to ergosterol, and is necessary for fungal cell membrane synthesis. All fungicides cause significant selection for resistance depending on their strength, dose and position in the crop canopy. Fluquinconazole controlled the disease best, but caused the strongest selection [Metcalfe *et al*., 2000].

The problem is worldwide and therefore the use of fungicides is only recommended for specific conditions. The Fungicide Resistance Action Committee (FRAG) publishes a long list summarizing current and historical resistance problems in the UK and potential problems in other countries [FRAG, 2007].

Besides the use of specific fungal chemicals and broadband pesticides, “soil disinfection” chemicals are applied, of which the most discussed is methyl bromide (MeBr). MeBr is an odorless, colorless gas used as a structural and soil fumigant in pest control for many years all over the world and across a wide range of agricultural sectors. The worst characteristic of MeBr is that it depletes the stratospheric ozone layer. Its strong negative impact motivated many EPA reports and commitments to reducing its use [EPA, 2008 a; Braun and Supkoff, 1994; USDA, 1996]. After a long struggle, its use was restricted and the

amount of MeBr in agriculture was reduced incrementally as required by the Montreal Protocol on Substances that Deplete the Ozone Layer (Protocol) and the Clean Air Act (CAA) [EPA, 2008 b]. Nevertheless, the battle against MeBr has not yet been won, and its worldwide use has not yet stopped [EPA, 2008 a; Braun and Supkoff, 1994; USDA, 1996]. Today the fight against MeBr has become one of the most important tasks for sustainable agriculture now and in the near future.

This thesis summarizes different approaches to sustainable, nontoxic water disinfection in agriculture, which hopefully can be applied in the near future. The new solar photocatalytic disinfection techniques will probably not be able to fully replace common pesticides and soil fumigants. But with the severity of the problems arising from the use of pesticides, any reduction in pollution by hazardous substances can be called a success for environmental protection, sustainability and human health.

II. 3. Hydroponic Agriculture: potential application for solar photocatalytic disinfection

A large area is already devoted to hydroponic agriculture worldwide [Jensen, 1989; Bussell *et al.,* 2004; Le Bot and Adamowicz, 2006]. Even in semiarid zones with high solar radiation, but scarce water sources, large commercial greenhouse operations making use of soilless cultivation are expanding, because they save water and fertilizer and optimize production [Jensen, 1989; 1991]. Soilless growing systems employ organic or inorganic inert artificial substrates based on perlite [Vazquez and Carpena-Ruiz, 2005], rock wool [Bussell *et al.,* 2004; Le Bot and Adamowicz, 2006] or coconut fiber [Raviv, 2005].

One of the highest expectations of hydroponics and soilless cultures is that they might serve as an alternative to methyl bromide soil fumigation [EPA, 2008 a; Braun and Supkoff, 1994; USDA, 1996]. Due to the strong environmental impact, this alternative is considered an important opportunity for sustainable agriculture.

Another advantage of soilless or hydroponic cultivation is increased productivity, as greenhouse planting densities can be doubled [Methyl Bromide Task Force 1995, Liebman 1994]. The hydroponic solution (nutrient-rich water) is usually pumped to the plants by

regulated drip irrigation systems. Wastewater from the roots is recaptured, disinfected, and reused. This practice not only helps reduce environmental waste and contamination, but conserves fresh irrigation water and controls nutrients [EPA, 2008; USDA, 1996; Liebman 1994].

Growers sterilize the recycled nutrient water by heating it to about 90 °C (194°F). Substrates are sterilized for reuse with steam [EPA, 2008; USDA 1996, Liebman 1994]. Even though power consumption is high with such heat sterilization systems, they are widely used. 90 ºC is high enough to inactivate many of the microorganisms, but complete sterilization would require even higher power (121°C and 1 bar overpressure for 15 min) applied to small volumes of water [Beth Israel Deaconess Medical Centre, 2004].

Sterilization of these liquid-nutrient solutions in hydroponic plantations is especially delicate, as soilless cultures develop a pathogenic environment in their plant root zone that is very different from traditional agriculture. Unfortunately, it can be said that the transition from soil to soilless cultures has not led to the disappearance of soil-borne diseases, but in the appearance of more water-borne diseases [Runia, 1994; Runia and Boonstra, 2001]. In this sense, phytopathogenic fungi are especially resistant pathogens, which spread easily in traditional plantations and even more quickly through pumped nutrient solutions [Gómez, 1993 and 2003; Sánchez *et al.,* 2004; Runia, 1994].

Various disinfecting agents have been tested for pathogen control, but as in the case of recirculating nutrient solutions, they have often turned out to be phytotoxic or hazardous to health or environment. Even chlorine and hydrogen peroxide soon show phytotoxicity [EPA, 2000; Jarvis, 1998; Coosemans, 1995]. As disinfection in soilless cultivation is especially sensitive to strong disinfecting agents, this nutrient solution is a good target for solar photocatalytic disinfection, which not only disinfects without introducing hazardous substances into the environment, but also becomes inactive in the dark root zones of the plants. This thesis demonstrates that disinfection of water containing phytopathogenic fungi by solar photocatalysis with TiO_2 (chapter 1) can be an alternative method for disinfecting nutrient solutions without using environmentally hazardous substances. The

synergistic effect of solar light and hydrogen peroxide at low concentrations is another option for this type of water disinfection. This issue is explored in chapter 5.

II. 4. Impact of *Fusarium* on agriculture

This work is most concerned with the fungus *Fusarium* as a plant pathogen in its various specialized forms. *Fusarium* is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes (organisms that absorb their nutrients from soluble organic compounds from decaying plant or animal matter). They are abundant members of the soil microbial community throughout the world [Summerell *et al.,* 1993; Sangalang *et al.,* 1995]. Some varieties of the species produce mycotoxins which can be harmful to human and animal health. The main toxins produced by these *Fusarium* species are fumonisins and trichothecenes [Agrios, 2004; Beckman, 1987].

As a fungus, *Fusarium* possesses a chitinous cell wall. The majority of fungal species are multicellular filaments, called hyphae, forming a mycelium. Some fungal species also grow as single cells. Sexual and asexual reproduction of the fungi is commonly via spores, often produced on specialized structures or in fruiting bodies. Some species have lost the ability to form specialized reproductive structures, and propagate solely by vegetative growth [Osiewacz, 2002].

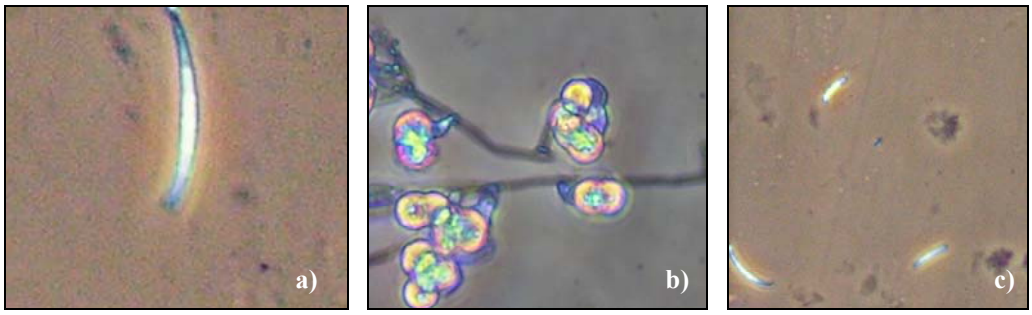


Figure 1 a) macroconidia **b)** chlamydospores **c)** macroconidia (left) and microconidia of *F. solani.*

Most *Fusarium* species are able to produce either two or all their spore types (**Figures 1 a-c**): microconidia, macroconidia and chlamydospores (increasing resistance, see chapter 2). These spores are highly resistant to environmental factors. It has been shown that

Fusarium graminearum macroconidia spores are able to germinate at temperatures from 4 to 20 °C, at pH 3-7 and after repeated freezing (-15° C) up to five times [Beyer *et al.,* 2004].

The genus also includes a number of important species pathogenic to plants. Its wide impact in all fields of agriculture makes this fungus a potent adversary with high impact on the present and future of agricultural industry and therefore on the nutrition of a major part of the earth's population. *Fusarium* species produce vascular withering, and root and stem rot in a wide variety of crops. Vascular wilt is a destructive disease of wide impact, which causes severe crop losses in many areas of the world [O'Donnell *et al*., 1998].

Its extent can be appreciated in the example of one of the most important diseases ever known in agriculture, "Panama disease", named after the country where it was first observed, or "Banana wilt". It is a devastating disease that attacked the first commercial banana variety, called "Grand Michel", introduced about 100 years ago. It is caused by *F. oxysporum* [Koeppel, 2007]. This disease was widespread in Asia, Africa, Australia, the Pacific Islands, the Caribbean, Central and South America, and wherever susceptible bananas, such as "Grand Michel" were cultivated [O'Donnell *et al*., 1998].

The *Fusarium* fungus invades young roots or plantroot bases, often through wounds. Early symptoms are a reddish-brown discoloration of the root xylem and then the rhizome. Eventually the stem is colonized and above ground symptoms appear. The older leaves are the first to turn bright yellow and wilt (**Figure 2 a, b**). As the disease progresses, it is always the youngest leaves that are affected until dead leaves surround the base. Affected plants eventually die after a few months [Koeppel, 2007].

As the land contaminated by Panama disease could not be reused, banana companies, in their fight against the disease, had to constantly occupy more and more land, which led to severe political and social problems. At last, in 1950, the banana companies ran out of room and at the last minute adopted the Cavendish banana, which was a less tastier variety, but resistant to Panama disease. The Grand Michel banana was thus made functionally extinct by a *F. oxysporum* strain around 60 years ago and today is found only in gardens

[Koeppel, 2007]. The fungus *Fusarium* was therefore able to functionally wipe out a whole plant species.

Figure 2 a) Young banana showing severe symptoms of Panama disease. The younger leaves show progressive yellowing, followed by necrosis (death), starting from the leaf margin. The older leaves have collapsed**. b)** Older banana plants showing advanced stages of Panama disease; note the "skirt" of dead leaves hanging down on each plant. Courtesy of Jim Deacon, The University of Edinburgh.

With the resistant Cavendish banana, which is in fact the banana we eat today in Europe and the USA, the problem seemed to be solved. Until around 10 years ago, when a new strain of Panama disease emerged and attacked Cavendish plantations in Malaysia. This new strain of Panama disease is a new resistant form of *F. oxysporum*, able to attack even the Cavendish. It spread even faster than the old one did and today the disease can already be found in India, Pakistan, China and Australia. It is estimated that in 5 - 10 years the entire banana variety could again be wiped out. Resistant banana species that might allow Europeans to keep eating bananas are quite different from the Cavendish banana we are used to. If the new strain of Panama disease can not be stopped by new genetic modification techniques, or soil and water disinfection, within 10 years we might have to get used to eating red bananas or pay high prices for the yellow one [Koeppel, 2007].

Another example of the impact *Fusarium* has on agriculture is its threat to coffee crops. Coffee is vital to the East and Central African economy, a major source of foreign currency and, as a “cash crop”, it is the livelihood not only of many people involved in its primary

cultivation, but also in its processing, marketing, and export. Since 1927 *Gibberella xylarioides* (*Fusarium xylarioides*), which causes "coffee wilt disease" (CWD) is responsible for huge coffee production losses in Africa. In the past it has been largely contained by a constant effort to find resistant plant varieties and often wide-scale plant protection practices. But a reemergence of CWD on coffee canephora (Robusta coffee) in Uganda, the Democratic Republic of Congo, and Tanzania has led to severe losses and, as in the case of the banana in Asia, future production in these regions of Africa is threatened. The relevance of CWD is acute and demands research to find a fast solution to this devastating disease [Rutherford, 2006].

Fusarium commonly infects barley when it rains late in the season, causing economic impact on the malting and brewing industries as well as feed barley. *Fusarium* contamination in barley can result in head blight and in extreme cases, barley can appear pink. *Fusarium graminearum* can also cause root rot and seedling blight. Total barley and wheat crop losses caused by *Fusarium* in the USA between 1991 and 1996 have been estimated at $3 billion [Priest and Campbell, 2002]. A more recent publication of Smily *et al*. reports estimations of wheat and barley crown rot in the Pacific Northwest of the USA, where this disease is caused by a *Fusarium pseudograminearum*, *Fusarium culmorum, Fusarium avenaceum, Bipolaris sorokiniana*, and *Microdochium nivale* complex. Increasing severity of crown rot caused reduction in grain yields of as much as 1,550 kg/ha (35%, $219/ha) in commercial fields [Smiley *et al*., 2005].

Vascular wilt attributed to *F. oxysporum* f. sp. *elaeidis* is also the most destructive disease in African oil palm causing severe losses in many areas. Over a period of 30 to 40 years, efforts to screen for resistance and continuous breeding programs in West Africa have helped to find more resistant oil palms and so to control the disease. But this effort must be continuous, with rigorous quarantine measures to prevent introduction of the pathogen in the highly productive areas of South East Asia (largest producer of palm oil) [Flood, 2006].

Symptoms include initial wilting followed by desiccation of the fronds, which finally break and hang around the trunk. Internally, characteristic browning of the vascular elements is seen both in adult palms and in seedlings. Two disease syndromes are commonly seen in

the field in adult palms, “acute wilt”, in which the palm dies within a few weeks and “chronic wilt”, in which the palm may remain alive for many months and even years, but becomes progressively stunted [Flood, 2006].

In the south of Spain, the *Fusarium* genus is also responsible for high losses in agriculture. In basil (*Ocimum basilicum L.*), *Fusarium* wilt was caused by *Fusarium oxysporum* f. sp. *basilica* with the typical symptoms of yellowing and wilting of apical tips, wilting and necrosis of leaves and petioles, stunting, black lines along the stems and petioles, vascular discoloration and necrosis of the xylem. Young plants die within 4 to 7 days after the first appearance of the symptoms. In the two areas evaluated in Almería, Spain, 14% of the plants were found to be infected [Guirado Moya *et al*., 2004]. Economically important diseases caused by *Fusarium* spp. in the region of Almería, Spain, have also been reported in tomato, melon, eggplant and peas [Tello and Lacasa, 1988; Gómez Vázquez and Tello, 2000; Urrutia Herrada *et al*., 2004; Jiménez Gasco *et al*., 2005].

There are also *Fusarium* species pathogenic in humans. In people with weak immune systems, these species may cause a range of opportunistic infections. In healthy people, fusarial infections may occur under the nails (onychomycosis) and infect the cornea of the eye (keratomycosis or mycotic keratitis), e.g., due to fungal contamination in contact lenses [Zhang *et al.,* 2007].

In humans whose immune systems are weakened in a particular way, aggressive fusarial infections penetrating the entire body and bloodstream (disseminated infections) may be caused by members of the *F. solani* complex, *F. oxysporum*, *F. verticillioides, F. proliferatum*, and rarely, other *fusarial* species [Howard, 2003]. In these patients, the skin is also often affected by *Fusarium* fungi, e.g. by *F. solani* [Gupta *et al.,* 2000]. These symptoms often result from chemotherapy for certain kinds of leukemia or heavy use of immunosuppressive drugs. Treatment is usually antifungal drugs like imidazole and triazole (DMIs), or Echinocandins, which inhibits the synthesis of glucan in the cell wall [Walsh and Dixon, 1996]. The present thesis investigates chances of other environmental friendly techniques for the disinfection of such pathogens. The investigated processes are

all based on the production of very reactive radical species and therefore part of the advanced oxidation processes.

II. 5. Advanced oxidation processes (AOPs) for water treatment

Advanced Oxidation Processes (AOPs) are characterised by the generation of hydroxyl radicals ($^{\bullet}OH$), the second strongest known oxidant, after fluorine (2.8 V vs. Standard Hydrogen Electrode, **Table 5**). It is therefore able to oxidize and mineralize almost any organic molecule yielding CO_2 and inorganic ions.

Table 5 Oxidation potential against Standard Hydrogen Electrode of some relevant oxidants [Legrini *et al.*, 1993; Kopecký, 1992].

Oxidant	Symbol	E^0 (V)
Fluorine	F_2	3.06
Hydroxyl radical	$^{\bullet}OH$	2.80
Singlet oxygen	$^1O_2$	2.42
Ozone	O_3	2.07
Hydrogen peroxide	H_2O_2	1.78
Perhydroxyl radical	$HO_2^{\bullet}$	1.70
Permanganate	MnO_4^-	1.68
Chlordioxide	ClO_2	1.57
Hypochloric acid	$HOCl$	1.45
Chlorine	Cl_2	1.36
Bromine	Br_2	1.09
Iodine	I_2	0.54

There are different techniques for generating hydroxyl radicals. The most important groups of AOPs are:

- Direct photolysis of oxidants (H_2O_2, O_3) or water with UV-C radiation [Legrini *et al.,* 1993; Gogate and Pandit, 2004 b].
- Heterogeneous photocatalysis [Legrini *et al.*, 1993; Gogate and Pandit, 2004 a; Herrmann, 1999].

- Fenton and Fenton-like processes with transition metals [Gogate and Pandit 2004 a, 2004 b; Safarzadeh-Amiri *et al.*, 1996].
- Cavitation techniques (hydrodynamic and ultrasound) [Gogate and Pandit, 2004 a, 2004 b].

The photochemical reactions (**Eq. 1 a-g**) for the generation of the hydroxyl radical in the most common AOPs involving radiation are given in **Table 6**.

Table 6 Hydroxyl radical generation by photochemical reactions [Pulgarín *et al.*, 1999; Safarzadeh-Amiri *et al.*, 1996; Legrini *et al*., 1993].

AOP	**Key reactions**	**(Eq. 1)**	**Wavelength**
UV/H_2O_2	$H_2O_2 + h\nu \rightarrow 2\,^{\bullet}OH$	**(a)**	$\lambda < 300$ nm
UV/O_3	$O_3 + h\nu + H_2O \rightarrow H_2O_2 + O_2$ $O_3 + H_2O_2 \rightarrow {}^{\bullet}OH + O_2 + HO_2^{\bullet}$	**(b)** **(c)**	$\lambda < 310$ nm
UV/H_2O_2/O_3	$O_3 + H_2O_2 + h\nu \rightarrow O_2 + {}^{\bullet}OH + HO_2^{\bullet}$	**(d)**	$\lambda < 310$ nm
UV/TiO_2	$TiO_2 + h\nu \rightarrow TiO_2 + (e^- + h^+)$	**(e)**	$\lambda < 390$ nm
Fenton Photo-Fenton	$Fe^{2+} + H_2O_2 + H^+ \longrightarrow Fe^{3+} + {}^{\bullet}OH + H_2O$ $Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + H^+ + {}^{\bullet}OH$	**(f)** **(g)**	$\lambda < 580$ nm

$^{\bullet}$OH radicals can react with organic compounds through abstraction of hydrogen or electrophilic addition to double bonds, generating free organic radicals ($R^{\bullet}$). These radicals react with oxygen molecules forming a peroxi-radical. So a series of degradation reactions can be initiated leading to total mineralization of organic compounds. The generation of highly reactive $^{\bullet}$OH radicals from H_2O_2 through the Fenton reaction has therefore attracted interest for treatment of nonbiodegradable residues in water treatment and soil-remediation [Pelizzetti *et al*., 1990 - 1995].

$^{\bullet}$OH radicals are characterized by their low selectivity of attack, meaning that they can be used for oxidation reactions with different compounds [Andreozzi *et al*., 1999 - 2000]. Total mineralization has been achieved for many pollutants such as halogenides, alkane

derivates, alkenes, carboxyl acids, aromatic substances, etc. [Pelizzetti *et al*., 1990-1995, Malato *et al*., 2002 a, b; 2007].

Production of UV radiation by lamps is expensive. Therefore, investigation is focusing increasingly on the two AOPs, which can be powered by solar radiation, i.e., light with a wavelength over 300 nm, which are the photo-Fenton reaction and heterogeneous catalysis by the UV/TiO_2 process [Bauer *et al.*, 1999; Malato *et al.*, 2002 a, b]. The first is known for its high reaction rates and the application of environmentally-friendly reagents (low-concentration iron salts, hydrogen peroxide decomposed to water and oxidising species consumed during treatment), which is why it could be considered one of the most promising photon-driven AOP for the remediation of contaminated water [Pulgarín *et al.*, 1999; Malato *et al*., 2007].

II. 6. TiO_2 photocatalytic processes

TiO_2 acts as heterogeneous photocatalyst between heterogeneous phases, e.g., solid/liquid or solid/gas. It is activated by absorption of wavelengths ($\lambda \leq 390$ nm) corresponding to its band gap energy E_G =3.2 eV. With this energy, electron-hole pairs are created in the semiconductor, which dissociate into photoelectrons in the conduction band and positive photo-holes (electron vacancies) in the valence band following **Eq. 2 a-c**.

$$TiO_2 + h \cdot \nu \rightarrow TiO_2 + (e^- + h^+) \qquad \textbf{(Eq. 2 a)}$$

$$A_{ads} + e^- \rightarrow A^-{}_{ads} \qquad \textbf{(Eq. 2 b)}$$

$$D_{ads} + h^+ \rightarrow D^+{}_{ads} \qquad \textbf{(Eq. 2 c)}$$

The electrons and holes created react with adsorbed molecules in the surrounding fluid (**Figure 3**). Only electrons and electron holes that reach the surface separately can react with an adsorbed donor or acceptor molecule. If this is not the case, separate charges can recombine within the TiO_2 particle (volume recombination of pairs) or on its surface (surface recombination of pairs). In aqueous media in the presence of oxygen, O_2 is usually the acceptor molecule (A) and water the donor molecule (D), as expressed in **Eq. 2 a-c**.

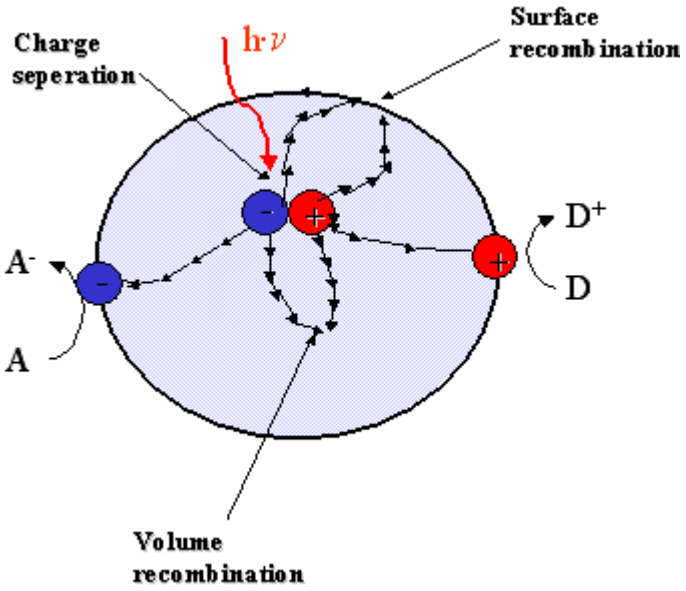


Figure 3 Charge separation and recombination within a spherical particle of TiO_2 under solar irradiation and in the presence of an acceptor (A) and donor (D) molecules [Gerisher, 1993, adapted].

The TiO_2 reaction depends mainly on the following steps:

1. Transfer of the reactants in the fluid phase to the (catalysts) surface.
2. Adsorption of at least one of the reactants
3. Reaction in the adsorbed phase (oxidative attack)
4. Desorption of the product(s)
5. Removal of the products from the interface region.

Therefore reaction kinetics is mainly governed by the following parameters: (i) catalyst mass, (ii) wavelength, (iii) partial pressure and/or concentrations of reactants, (iv) temperature and (v) the radiant flux [Herrmann, 1999, 2005].

These parameters have been studied in detail for the degradation of an enormous number of chemical contaminants in water [Augugliaro *et al*., 2006; Fujishima *et al*., 2000]. The basic reactions that lead to the generation of $^{\bullet}OH$ radicals can be separated into reactions that occur at the reduction and oxidation sites. The radical generation summarized here (**Eq. 3 and 4**) can be further subdivided into various reaction steps and accompanied by co-reactions depending on the catalysts surface and the molecules present in the water,

which have been thoroughly described in the literature [Hoffmann, 1995; Herrmann, 1999, 2005].

Reduction site:

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad \textbf{(Eq. 3 a)}$$

$$O^{\bullet -}_2 + H^+ \rightarrow HO_2^{\bullet} \quad \textbf{(Eq. 3 b)}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad \textbf{(Eq. 3 c)}$$

$$H_2O_2 + e^- \rightarrow OH^- + {}^{\bullet}OH \quad \textbf{(Eq. 3 d)}$$

where (A) is the acceptor molecule (O_2) is oxygen and e^- is the electron set free in the conduction band.

Oxidation site:

$$H_2O + h^+ \rightarrow {}^{\bullet}OH + H^+ \quad \textbf{(Eq. 4 a)}$$

$${}^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad \textbf{(Eq. 4 b)}$$

$$H_2O_2 + 2h^+ \rightarrow 2H^+ + O_2 \quad \textbf{(Eq. 4 c)}$$

where h^+ is the hole photo-generated in the valence band of the semiconductor with water (H_2O) as the donor molecule (D).

II. 7. Photocatalytic disinfection with TiO_2

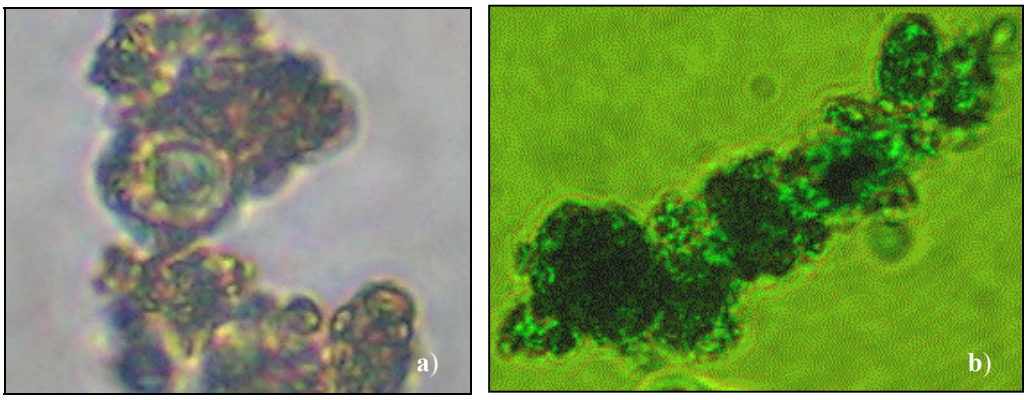
Since 1985, the field of TiO_2 disinfection research has been growing faster and faster. Literature to 1998 is summarized in the detailed review by Blake *et al*., including medical applications [Blake *et al*., 1999]. From 1985 to date, more than 160 peer reviewed articles have been published only in the field of TiO_2-assisted water disinfection for a wide range of microorganisms (www.scopus.com, 2008).

The first reports on the potential of TiO_2 for disinfection was by Matsunaga *et al*. in 1985. These authors showed inactivation of the Gram-positive bacteria *Lactobacillus acidophilus*, the Gram-negative bacteria *E. coli*, the yeast *Saccharomyces cerevisiae* and the algae *Chlorella vulgaris* after 120 min of incubation with irradiated TiO_2/Pt powders.

Inactivation from 10^3 CFU/mL to the detection limit was reported, except for the alga, which after 120 min still showed 55% survival. Due to the differences in cell wall properties of the microorganisms tested and independent tests with coenzyme A, the authors attributed disinfection to photochemical oxidation of the intracellular coenzyme [Matsunaga *et al.*, 1985]. This initial publication was later extended by the same group to application of TiO_2 powder immobilized on acetylcellulose membranes in a continuous sterilization system [Matsunaga *et al*., 1988].

Later publications investigated the exact bactericidal mechanism of TiO_2 photocatalysis. The cell wall is thought to be the first site of attack by the reactive hydroxyl radicals. "Rapid" leakage of potassium ions from the bacteria parallel to the decrease in cell viability was reported by Saito *et al*. [Saito *et al.,* 1992]. Maness reported results that can be explained by peroxidation of the polyunsaturated phospholipid component of the cell membrane leading to a loss of essential cell functions, e.g., respiratory activity, and in the end, to cell death [Maness *et al.*, 1999].

In the following years, several publications reported cell wall disruption and leakage of contents after UV/TiO_2 disinfection treatment [Nadtochenko, 2004; Kiwi and Nadtochenko, 2004; Sunada *et al.,* 2003]. *E. coli* photokilling was followed by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) and atomic force microscopy (AFM) by Nadochenko *et al*. These techniques allowed them to determine the formation of peroxidation products due to photocatalysis of *E. coli* cells, thereby providing even stronger evidence that the changes in the *E. coli* cell wall membranes are precursor events leading to bacterial lysis [Nadtochenko, 2004; Kiwi and Nadtochenko, 2004]. A very recent publication on this subject studies the adsorption of *E. coli* K 12 cells onto TiO_2 particles (1 g/L) over 60 min of illumination in sodium phosphate solutions. In this article, the rate of adsorption of bacteria onto TiO_2 is reported to be positively correlated with the bactericidal effect of TiO_2. Flow cytometry confirmed that bacterial adsorption could be consistently associated with a reduction or complete loss of *E. coli* membrane integrity [Gogniat *et al*., 2006].



Figures 4 a), b) Chlamydospore (left) and macroconidia (right) covered with TiO_2 after 5 h of the photocataltytic disinfection treatment.

The cell wall is more and more accepted as the first target of TiO_2 disinfection [Malato *et al*., 2007]. In the case of *Fusarium* the interaction between spores and the catalyst is very strong. But it can be observed that instead of fungi adsorbing onto TiO_2 particles, it is actually the TiO_2 particles that adsorb onto the fungal spores (**Figures 4 a, b**).

Ireland *et al*. (1993) reported on disinfection of pure cultures of *E. coli* with anatase crystalline TiO_2 in a flow-through water reactor. Their work already had the foresight to use TiO_2 for drinking water disinfection. They used dechlorinated tap water and surface water samples to evaluate its disinfection capability, and reported rapid cell death for both pure cultures and members of the indigenous flora of natural water samples [Ireland *et al*., 1993]. This forward-looking approach probably made their work one of those with the most impact and citations in TiO_2 disinfection.

Over the course of time, work with better and better disinfection yields have been published, reporting a four-order decrease in *E. coli* concentration in TiO_2 suspensions exposed to sunlight for 23 min in a batch reactor [Zhang *et al.*, 1994]. Pham *et al*. reported a 95% reduction of *B. pumilus* spores after exposure to UV light ($\lambda = 365$ nm) in the presence of suspended TiO_2 in water [Pham *et al*., 1995]. Also the inactivation of several common bacteria (*Serratia marcescens, E. coli,* and *Streptococcus aureus*) with UV-C lamps or sunlight was reported in less than 10 minutes in the presence of TiO_2 powder [Block *et al*., 1997; Bekbölet, 1997].

Little by little, TiO_2 disinfection research went from basic laboratory studies to the first trials with real disinfection applications. In 2000, Herrera Melian *et al*. reported on TiO_2-assisted disinfection of urban waste water. Two microbial groups, total coliforms and *Streptococcus faecalis*, were disinfected employing both an UV-C-lamp and solar light. This publication was also one of the first to report difficulties with TiO_2 disinfection applied to real water, finding very little difference between TiO_2-photocatalysis and direct solar or UV-C-lamp light irradiation at natural sample pH (7.8), while at pH 5 the presence of TiO_2 notably increased the relative inactivation rate [Herrera Melian *et al*., 2000]. On the other hand Rincón and Pulgarin did not find any modification in the inactivation rate of *E. coli* in distilled water due to changes in initial pH between 4.0 and 9.0 in the absence or presence of TiO_2 under simulated sunlight illumination [Rincón and Pulgarin, 2004 a].

Herrera Melian *et al*. also published the first mention of a positive effect of TiO_2 disinfection after a long residence time of two days, when compared to bare UV or solar irradiation [Herrera Melian *et al*., 2000]. This issue of improved effect after long time dark periods (probably due to better disinfection yield at the beginning) is discussed in many publications. Of course TiO_2 loses activity in the darkness but authors refer to the absence of bacterial growth. Wist *et al.* reported the lack of a residual effect due to strong bacteria growth (regrowth) in treated water from the Cauca River (Cali, Colombia) after 24 h as a disadvantage of TiO_2 disinfection of *E. coli* [Wist *et al*., 2002]. However, Rincón and Pulgarin reported a light-intensity dependent "residual disinfecting effect" in the presence of TiO_2 (400 or 1000 W/m^2) compared to solar-only disinfection of *E. coli* K12 and bacteria consortia, and failed to observe regrowth within the 60 h following treatment with TiO_2 [Rincón and Pulagrin, 2004 b].

Microorganisms that are very resistant to UV-A irradiation like *Enterobacter cloacae* have been successfully inactivated by TiO_2 photocatalysis [Ibañez *et a*l., 2003]. The authors further report the successful inactivation of various Gram-negative strains of bacilli with differing photosensitivity, such as *E. coli*, *P. aeruginosa* and *S. typhimurium*.

Recent TiO_2 disinfection research focuses more on disinfection applied to more resistant microorganisms. Seven *et al*. successfully inactivated *E. coli*, *P. aeruginosa*, and

Staphylococcus aureus, within 40 min and of *S. cerevisiae*, *C. albicans,* within 120 min in the presence of TiO_2, ZnO and Sahara desert sand under lamp irradiation. The very low catalyst concentration they used for the TiO_2 slurry is worth notice: 0.01 g/L. This paper also reports that the only filamentous fungus tested, *Aspergillius niger*, was resistant to the treatment described [Seven *et al.,* 2004].

Lonnen *et al*. confirm inactivation of *C. albicans* by TiO_2 photocatalysis and report a 5.5 log decrease in *F. solani* after 4 h of simulated sunlight. They were the first group to publish such high inactivation rates with a fungal test organism, and especially, with supported TiO_2 [Lonnen *et al*., 2005]. But it should be mentioned that their 200 W/m^2, 300 – 400 nm UV Xenon lamp provided much stronger energy than would be expected under real sunlight (maximum solar UV-A irradiation at the PSA, Spain: 55 W/m^2).

Besides demonstrating the susceptibility of very resistant microorganisms, the TiO_2 disinfection treatment process has been chemically characterized and optimized for engineering. The work of Rincón and Pulgarin should be specially mentioned for their complete and very detailed characterization of chemo-physical factors influencing the photocatalytic TiO_2 disinfection process for *E. coli* and other bacteria. Among other factors, the influence of different types of bacteria consortia, water sources, TiO_2 catalyst type, slurry TiO_2 compared to supported catalyst, UV-light dose, etc., can be found in their work [Rincón and Pulgarin, 2001-2005].

Apart from direct water disinfection applications, TiO_2 has been used with chlorine to decrease the concentrations of disinfection by-products (DBPs) in chlorine disinfection. In 1996, Richardson *et al*. reported only one organic DBP (tentatively identified as 3-methyl-2,4-hexanedione) in ultra filtered raw water treated with TiO_2/UV, while several chlorinated and brominated DBPs were formed, among them some halomethanes and several halonitriles, when chlorine was used as a secondary disinfectant, following TiO_2/UV treatment. The number and concentration of these chlorinated DBPs was still significantly lower than when chlorine was used as the sole disinfectant [Richardson *et al*., 1996].

Rincón *et al.* also showed positive effects of the presence of TiO_2 on the removal of DBPs in water. Complete *E. coli* inactivation by simulated solar irradiation of a TiO_2 suspension within 20 min, and 70 min with light alone was reported. The addition of disinfection by-product precursors (DBPPs), like dihydroxybenzenes, e.g., resorcinol, hydroquinone and catechol, resulted in a decrease in germicidal activity, but in the end a combined disinfection and decontamination method could be achieved [Rincón *et al*., 2001].

The biggest problem with TiO_2 slurry disinfection was soon recognized to be the need for post-treatment TiO_2 recovery. Contrary to small concentrations of chlorine, TiO_2 powder cannot be left in drinking water due to inacceptance and insufficiently assessed health risks. Therefore, much research was done to yield efficient catalysts on supports that would keep the TiO_2 out of the treated water. Unfortunately, in photocatalytic disinfection, almost all immobilized TiO_2 either had very limited yields or involved technical effort leading to high cost. It was even reported that immobilization of TiO_2 affects the disinfection of *E. coli* more than chemical contaminant degradation, e.g., gallic acid [Gumy *et al*., 2006].

One of the earliest reports on this topic was published in 1997 for a TiO_2 film reactor and a combination of UV light and an electric field to disinfect water containing *Clostridium perfringens* spores and *E. coli* [Butterfield *et al*., 1997]. In 2002, Curtis *et al*. reported damage to the pathogen *C. parvum,* responsible for many human diseases [Robertson *et al.,* 1992; Slifko *et al.,* 2000]. They disinfected *C. parvum* oocysts in electric-field-enhanced photo-oxidation using immobilized titanium dioxide. In simple Petri dish reactors, two forms of immobilized titanium catalyst (sol-gel and thermal-film) the increase in *Cryptosporidium* oocyst permeability was assessed by propidium iodide exclusion. The results showed that oocyst permeability increased approximately 27% with the thermal-film, and was even less effect with the sol-gel film [Curtis *et al.*, 2002]. Much better inactivation results for *C. parvum* were achived by Méndez-Hermida *et al*. with TiO_2 fixed on flexible plastic inserts in 1.5-mL bottles under natural sunlight [Méndez-Hermida *et al.,* 2007].

To improve efficiency of the immobilized photo-catalysts, some work has been done in electric-field to promote photoelectrocatalytic disinfection. For example, TiO_2 powder can be immobilized electrophoreticaly on electrodes. When electric fields were applied to

TiO_2, *E. coli* K12 disinfection rates were reported to increase by 40% using Degussa P25 electrodes and by 80% using Aldrich electrodes [Dunlop *et al*., 2002]. A similar publication by Christensen *et al*., reported the photoelectrocatalytic and photocatalytic disinfection of *E. coli* suspensions by titanium dioxide in a sparked photo electrochemical reactor with "thermal" electrodes (oxidation of titanium metal mesh) and "sol-gel" electrodes (depositing and then heating a layer of titania gel on titanium mesh). The authors reported that the photoelectrochemical system with "thermal" electrodes was more efficient than their photocatalytic system with TiO_2 slurry [Christensen *et al*., 2003].

Another way to increase the efficiency of TiO_2 coatings is to modify their chemical composition by doping with other elements, e.g., iron, silver, copper [Armelao *et al*., 2007; Ditta *et al*., 2008]. Yu *et al*., for example, took advantage of the diffusion of iron atoms through TiO_2 dipcoatings placed on stainless steel. Due to this diffusion of Fe^{3+} and Fe^{2+} ions from stainless steel substrate into TiO_2 films during high-temperature calcinations, the iron behaves as a dopant and the results show significantly better activity than those coatings on glass. In addition, this TiO_2 film seemed to show photo-induced hydrophilicity and so could be used for the sterilization of *Bacillus pumilus* [Yu *et al*., 2003].

In one of the most important publications on the use of TiO_2 nanoparticles in disinfection, in 2005, Yu *et al*. even reported positive results for doping with non metals. The authors found that sulphur-doped TiO_2 nanoparticles showed strong visible-light-induced activity that effectively kills *Micrococcus lylae*, a common Gram-positive bacterium, within 1 h on S-doped TiO_2 under visible light irradiation [Yu *et al*., 2005]. In the last few years, nanotechnology (manipulates nanoscale matter 1-100 nm) has itself become a field in TiO_2 photocatalysis for disinfection and water treatment in general [Armelao *et al*., 2007; Ditta *et al*., 2008; Theron *et al*., 2008].

Even though these promising achievements in TiO_2 by materials science show many possible future applications, to date, the most positive results for water decontamination and disinfection have been reported for the Degussa P25 anatase-rutile powder TiO_2 [Herrmann, 2005; Malato *et al*., 2007].

Gumy *et al*. tested the photocatalytic activity of different types of TiO_2 catalyst (Degussa P-25, Millennium PC-100 and PC-500, Tayca AMT-100 and AMT-600) in suspension or coated on a fibrous web in laboratory decontamination and disinfection experiments. They found good disinfection capacities for all tested catalysts in suspension [Gumy *et al.*, 2006 a].

Gumy *et al*. later changed the pH of the disinfection solution and demonstrated that the good photocatalyitc disinfection capacity for TiO_2 P25 did not vary over a wide pH range (3 - 7.5). Other TiO_2 photocatalysts lost their activity with increasing pH, depending on their isoelectric point (IEP). The surface charge of the catalyst changes from positive at pH < IEP to negative for pH > IEP, which probably affects the interaction between the catalyst surface and the microorganism, since cell walls of microorganisms have slightly negative surface charges [Szumski *et al*., 2005; Girardin *et al*., 1999]. Thus, the lower the TiO_2 IEP, the lower the bacterial inactivation observed [Gumy *et al*., 2006 b].

Another critical issue in disinfection is the evaluation of the solar radiation standardization parameters used for photocatalytic treatment. Rincón and Pulgarin designed the first method of assessing the bactericidal inactivation rate in solar photocatalytic processes for drinking water [Rincón and Pulgarin, 2004 c]. They demonstrated that the solar UV dose necessary to reach target disinfection levels can not be the only indicator of system efficiency, and proposed a new parameter called, "effective disinfection time" (EDT), defined as the treatment time necessary to avoid bacterial regrowth after 24 (or 48 h) in the dark after phototreatment. They published a detailed study about the specific effect of residence time of water in the illuminated part of the solar disinfection system, light intensity and the time of day (morning or afternoon) exposed to solar radiation, and not only the total photon dose. Their publication gave directly motivated the work presented in chapter 4 of this thesis, "Effect of UV-intensity and dose on the photocatalytic disinfection of bacteria and fungi".

II. 8. Detrimental effects of light on microbial cells

The very energetic solar UV-C (220-290 nm) band is absorbed by the atmosphere, and is therefore not a component of the sunlight responsible for such phenomena as skin cancer. UV-C light (used e. g. in disinfection lamps), when absorbed by the cell DNA (**Figure 5**), damages irradiated DNA, directly inducing pyrimidine and purine dimers [Bose *et al.,* 1983] and pyrimidine adducts [Brash *et al.,* 1982].

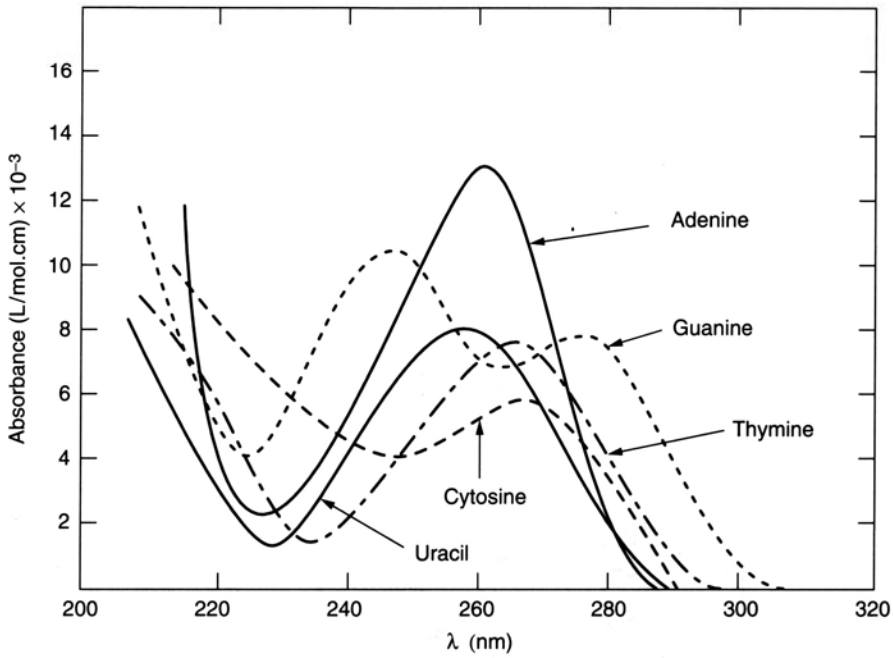


Figure 5 Absorption of pyramidine bases in the UV- range [Masschelein, 2002].

The solar ultraviolet radiation that reaches the surface of the earth contains UV-B (290 - 320 nm) and UV-A (320 - 380 nm) light. In this wavelength range, only the UV-B region overlaps with the tail of DNA absorption (**Figure 5**) [Masschelein, 2002]. UV-B radiation is believed to be the component of sunlight mainly responsible for the majority of human skin cancers [Tyrrell and Keyse, 1990]. Nevertheless, the UV-A region of sunlight is potentially carcinogenic and is certainly involved in photoaging, so much research has been undertaken to understand the cell damaging action of both components (UV-A and UV-B) [Tyrrell and Keyse, 1990].

Today it is accepted that the damage caused by UV-A and UV-B light is mainly due to its absorbtion by cellular components called intracellular chromophores [Favre, 1977; Tyrell and Keyse, 1990; Moan and Peak, 1989; Black *et al.,* 1997; Ravanat *et al.,* 2001].

The best known intracellular chromophore is probably L-tryptophan [McCormick *et al*., 1976], which after irradiation produces photoproducts that showed toxicity on recombinationless (rec) mutants of *Salmonella typhimurium* [Yoakum and Eisenstark, 1972] and on *E. coli* [Yoakum *et al.*, 1974 and 1975]. δ-aminolevulinic acid was also detected as an intracellular chromophore, after it was shown that *E. coli* mutants, defective in the synthesis of δ-aminolevulinic acid, are resistant to UV-A inactivation [Tuveson and Sammartano, 1986]. Potential chromophores are also considered to contain unsaturated bonds such as flavins, steroids and quinines [Tyrell and Keyse, 1990].

The irradiation of intracellular chromophores with UV-A light is only toxic to cells in the presence of oxygen [Peak *et al.,* 1985; Moan and Peak, 1989; Tyrrell and Keyse, 1990]. Damage by light absorption through chromophores is therefore contributed to the generation of reactive oxygen species (ROS). The resulting oxidative stress damages the cells and cell components [Favre, 1977; Tyrell and Keyse, 1990; Moan and Peak, 1989]. Besides other cell damage, ROS can lead to lipid peroxidation [Vile and Tyrrell, 1995], pyrimidine dimer formation [Tyrrell, 1973] and even DNA lesions [Imlay and Linn, 1988].

ROS can be inhibited by scavenging enzymes, such as catalase, peroxidase and superoxide dismutase, which are found in all aerobic organisms [Fridovich, 1986]. Scavenging agents not only act during UV-A-irradiation-induced oxidative stress, but also during normal cell life to correct oxidative lesions during the cell metabolism itself [Demple and Harrison, 1994]. These oxidative lesions can be caused during cell respiration by three active intermediates of the univalent reduction of molecular oxygen to water: Peroxylradical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) [Fridovich, 1986].

While intracellular UV-A absorption induces the superoxide radical directly [Cunningham *et al.,* 1985], the intracellular $^{\bullet}OH$ radical formation can be attributed to the

Fenton and Haber-Weiss reaction [Minotti and Aust, 1984; Mello Filho and Meneghini, 1987].

II. 9. Fenton processes in water treatment

The Fenton reaction (**Eq. 5 a**) is known as the iron dependend, univalent reduction of H_2O_2 postulated by Fenton [Fenton, 1894]. This reaction occurs in the absence of light and preferably in acidic media under the production of $^{\bullet}OH$ radicals The regeneration of Fe^{2+} ions follows different path as resumed in **Eq. 5 b** and **Eq. 5 c** and **Eq. 5 d** known as the Haber- Weiss cycle [Haber and Weiss, 1943; Walling, 1975]:

$$Fe^{2+} + H_2O_2 + H^+ \longrightarrow Fe^{3+} + {}^{\bullet}OH + H_2O \qquad \textbf{(Eq. 5 a)}$$

$$Fe_{aq}{}^{3+} + H_2O_2 \longrightarrow Fe_{aq}^{2+} + HO_2^{\bullet} + H^+ \qquad \textbf{(Eq. 5 b)}$$

$$^{\bullet}OH + H_2O_2 \longrightarrow HO_2^{\bullet} + H_2O \qquad \textbf{(Eq. 5 c)}$$

$$HO_2^{\bullet} + Fe^{3+} \longrightarrow O_2 + Fe^{2+} + H^+ \qquad \textbf{(Eq. 5 d)}$$

Hydroxyl radical generation from H_2O_2 only takes place in presence of reactive transition metals. Iron (II), along with H_2O_2, called Fenton's reagent, catalyses the Fenton reaction.

Under appropriate irradiation (UV-Vis), the Fenton reaction can become the photo-Fenton reaction. As iron absorbs in the energetic part of the sunlight under UV-Vis illumination ($\lambda \leq 550$ nm), Fenton reaction rates strongly increase, because of the higher concentration of the Fenton catalyst, Fe^{2+}, by the photochemical reduction of Fe^{3+} **(Eq. 5 e)** [Pignatello, 1992; Rupert *et al*., 1993].

$$H_2O + Fe^{3+} + h\nu \longrightarrow Fe^{2+} + {}^{\bullet}OH + H^+ \qquad \textbf{(Eq. 5 e)}$$

Photo-Fenton reactions depend not only on the concentrations of iron and H_2O_2 but also on light intensity. Today, not only the Fenton, but also the photo-Fenton process, are well characterized and already used in many applications for a great number of hazardous substances [Pignatello and Sun, 1995; Chiron *et al.,* 2000; Gogate and Pandit, 2004]. The

process parameters and applications summarized above have been recently reviewed in detail [Malato *et al.,* 2002 a, b; Pera-Titus *et al.,* 2004; Malato *et al*., 2007].

The hydroxi-ferric complexes, produced during the Fenton reaction, easily coagulate and precipitate between pH 3 and 7. This is avoided at pH < 3 using optimized amounts of H_2O_2 [Malato *et al*., 2007]. This means that for any kind of wastewater treatment the water first has to be adjusted to pH 3 or lower.

Reactions that take place at higher pH are called Fenton-like reactions and the iron is kept in the reaction by complex-forming organic ligands. These Fenton-like reactions have already been used successfully in the treatment of organic contaminants to increase the pH range of the Fenton reaction [Georgi *et al*., 2007; Lauren *et al*., 2006].

The problem with using Fenton-like processes in water disinfection and water treatment in general is the competition for $^{\bullet}OH$ radicals between the contaminant and the ligands, which maintain the iron in solution. Once the organic ligands are oxidized, the iron is no longer held in solution at neutral or near neutral pH. The need for low pH for reactions has also been a strong criticism of Fenton processes in disinfection. At pH around 3, most microorganisms are no longer viable without the need of further treatment.

The only recent work about Fenton treatment for disinfection was published by Rincón and Pulgarin for *E. coli* [Rincón and Pulgarin, 2005 a]. The authors used real water from Lemans Lake in Switzerland, concentrations of 10 ppm iron from iron salts and 10 ppm H_2O_2 at neutral pH. Their paper reports positive disinfection results, although they do not monitor the iron or H_2O_2 concentrations. It is therefore difficult to know how much iron remains in solution at oxygenated and neutral pH conditions and in what manner this iron is capable of catalyzing the Fenton reaction. In chapter 5 below, it is suggested that under solar irradiation in presence of H_2O_2, Fenton- catalyzed disinfection already takes place, without the addition of iron.

II. 10. Intracellular iron control and oxidative stress

In the normal metabolism of aerobic cells, H_2O_2 is one of the metabolites originating from the catalyzed reduction of oxygen to water during cell respiration [Fridovich, 1986]. According to the Fenton reaction, H_2O_2 and iron released inside the cells can cause damage to cell functions *in vivo* and *in vitro* by catalyzing the production of reactive oxygen radicals [Filho and Meneghini, 1987; Imlay *et al.,* 1988; Keyer and Imlay, 1996]. The importance of the presence of iron to the initiation of lipid peroxidation was also shown by Minotti and Aust [Minotti and Aust, 1987].

Even if iron is a potentially dangerous element for living organisms, it is still vital to cell structure, which is why aerobic cells have developed methods to control their intracellular iron levels [Andriopoulos *et al.*, 2007; Varghese *et al.*, 2007; Tyrrell and Reeve, 2006]. In mammals, iron is stored in macromolecules as ferritin. From these molecules, iron can be recovered as required by the cell. The amount and form in which iron is present in cells has been discussed in many scientific papers reviewed in a recent publication of Kakhlon and Cabantchik [Kakhlon and Cabantchik, 2002].

In 1976, Jacobs proposed the cellular labile iron pool (LIP). This LIP is a model for cell iron in its different forms not captured in the storage molecules [Jacobs, 1976]. It was operationally defined as a cell chelatable iron pool that may be a mixture of both, iron II and iron III [Vile and Tyrrell, 1993]. The more iron is chelatable, the more available it becomes for Fenton reactions, and therefore, cell damage [Minotti and Aust, 1984]. The control of the intracellular iron concentration is very complex and now seems to be easily disrupted by oxidative stress.

Pourzand *et al*. reported recently, that UV-A irradiation of skin cells can change intracellular iron levels, leading to immediate release of labile iron, possibly from ferritin in human skin fibroplasts [Pourzand *et al.,* 1999; Vile and Tyrrell, 1993]. How and why oxidative stress changes intracellular free iron levels in prokaryotic and eukaryotic cells and where iron is released from, have been issues in a great number of important scientific articles [Vile and Tyrrell, 1995; Pourzand *et al.,* 1999; Cadet *et al.,* 2005; Andriopoulos *et al.,* 2007; Varghese *et al.,* 2007; Tyrrell and Reeve, 2006].

Recently, more and more publications explain increased iron in cells after oxidative stress (e. g. induced by UV-A challenge) by reactions between ROS and iron-sulfur clusters of iron-regulating proteins like Fur or m-aconitase. ROS attacks on oxidative-stress-sensitive proteins can directly liberate an iron atom from the cluster or indirectly increase the iron levels, causing changes in transcriptional iron regulation [Varghese *et al.,* 2007; Beinert *et al.,* 1996; Kiley and Beinert, 2003].

II. 11. The lethal synergy of H_2O_2 and near-UV light

The lethal synergy of H_2O_2 and near-UV light was first reported by Anathaswamy *et al.,* for phage T7 [Anathaswamy *et al.,* 1979; Anathaswamy and Eisenstark, 1977]. The same group published a follow-up paper in 1980 on the combined lethality of H_2O_2 and near UV-radiation for *E. coli* K12 [Hartman and Eisenstark, 1980]. Both publications attribute the increased lethality to augmented oxidative stress from the creation of a new chromophore, a photoproduct originating from irradiation of L-tryptophan [McCormick *et al.*, 1976; Hartman and Eisenstark, 1980]. They explain that the new L-tryptophan photoproduct was toxic to recombinationless (rec) mutants of *Salmonella typhimurium* [Yoakum and Eisenstark, 1972] and of *E. coli* [Yoakum *et al.*, 1974 and 1975].

There is practically no further literature on the lethal synergy of H_2O_2 and artificial UV-A or natural sunlight. One of the few papers citing the work of Hartman and Eisenstark was published by Rincón and Pulgarin's Group. They report increased *E. coli* inactivation under solar radiation in the presence of H_2O_2, but this increased H_2O_2 inactivation is only a blank test for photo-Fenton experiments with *E. coli* disinfection. Rincón and Pulgarin also use the interpretation of Eisenstark, or simply "greater sensivity of *E. coli* to solar disinfection" as the explanation for the increased bacterial inactivation observed [Rincón and Pulgarin, 2005 a].

To date, this lethal synergy has never been discussed in the context of the Fenton reaction after iron up-regulation following UV-A-induced ROS attacks. Nevertheless, the results reported in chapter 5 on increased lethality of H_2O_2 (at low concentrations) by sunlight will be discussed with the help of the abundant recent literature. As radical production in combination with sunlight and iron is closely connected to skin cancer, and therefore, the

medical field, the topic is of great interest and many studies have been published in the last few years [Varghese *et al.,* 2007; Beinert *et al.,* 1996; Kiley and Beinert, 2003]. In chapter 5, this synergic inactivation of fungal cells is also attributed to generation of $^{\bullet}OH$ radicals from H_2O_2 after the Fenton reaction.

If synergy of light and H_2O_2 exists in presence of intracellular iron, why are we discussing the Fenton, and not the photo-Fenton, reaction? As explained above, the solar light in the photo-Fenton process contributes to redox cycling of Fe^{3+}–Fe^{2+}in the presence of H_2O_2 and at acid pH. But the literature on intracellular iron control only proves that increased iron concentrations exist in near UV-irradiated cells, not that the intracellular iron is further reduced from Fe^{3+} to Fe^{2+} by sunlight. The analytical tools available for this thesis did not allow to measure increased intracellular Fe^{2+} concentrations. Therefore, the results of chapter 5 are discussed as a consequences of a Fenton-based generation of $^{\bullet}OH$ radicals, not a direct photo-Fenton process. Even if influence of the photo-Fenton process on fungal inactivation, is not excluded. Chapter 5 is also the first work to report the existence of a lethal synergy of Hydrogen Peroxide and near-UV radiation to fungal spores, leading to a good opportunity for water disinfection for agricultural applications.

II. 12. Solar Disinfection

The first successful application of sunlight to drinking water disinfection was published in 1984. Acra *et al*. used sunlight for disinfection of oral rehydration solutions brought to developing countries as part of the World Health Organization (WHO) disease control program [Acra *et al.,* 1984].

Since then, the inactivation of a large part of microorganisms, including bacteria [Joyce *et al.,* 1996, McGuigan *et al.,* 1998, Berney *et al.,* 2006 a], cysts [Heaselgrave *et al.,* 2006; McGuigan *et al.,* 2006] and fungi [Lonnen *et al.,* 2005; Sichel *et al.,* 2007 a] has been reported (**Table 7**). Resistance of the microorganisms present to solar disinfection leads to variation in treatment times [Berney *et al.,* 2006 a; Sichel *et al.*, 2007 a; McGuigan *et al.,* 20006]. Even the growth phase of the microorganisms influences their susceptibility to disinfection [Berney *et al.,* 2006 b].

Under specific conditions, drinking water is already treated by solar disinfection, or SODIS, as mentioned further below. The term SODIS refers particularly to small household solar disinfection in polyethylene terephthalate (PET) bottles [Wegelin *et al.,* 1994], and has already been tested successfully in real field applications e.g., in Kenya Masai communities [Conroy *et al.,* 1996] as well as in many other projects worldwide [EAWAG-SANDEC].

Table 7 Inactivation times of microorganisms in SODIS process.

Microorganism	**Inactivation time under approx. 1000 W/m^2 global irradiance**	**Reference**
Bacillus subtilis endospores	No inactivation after 8 h	[Boyle *et al.*, 2008]
Yersinia enterocolitica	3 h	[Boyle *et al.*, 2008]
Enterpathogenic E. coli	1.5 h	[Boyle *et al.*, 2008]
Staphylococcus epidermis	35 min	[Boyle *et al.*, 2008]
Campylobacter jejuni	20 min	[Boyle *et al.*, 2008]
Fusarium solani	5 h	[Sichel *et al.*, 2007 a]
Fusarium oxysporum	5 h	[Sichel *et al.*, 2007 a]
Cryptosporidium parvum	8 h	[Méndez-Hermida *et al.,* 2007]
Giardia muris cysts	4 h	[McGuigan *et al.*, 2006]
*Acanthamoeba polyphaga (*cysts)	6 h	[Heaselgrave *et al.,* 2006]
Polio virus (NCPV #503)	6 h (40 °C)	[Heaselgrave *et al.,* 2006]
A. Polyphaga (Trophozoites)	6 h	[Lonnen *et al*., 2005]
Escherichia coli DH5 a	2.5 h	[Lonnen *et al*., 2005]
Pseudomonas aeruginosa	2 h	[Lonnen *et al*., 2005]
Candida albicans	6 h	[Lonnen *et al*., 2005]

Even if the results of SODIS are very promising, it is only used for disinfection in remote low-income areas without access to clean drinking water. In industrialized areas with fewer

economic limitations, ozone, UV-C and chlorine disinfection are used. The effectiveness of the process depends on the original water quality, temperature, turbidity, and resistance of the specific microorganisms, irradiance and dissolved oxygen [Kehoe *et al.,* 2000]. Over 45 °C, a there is synergy between thermal heating and solar UV inactivation which leads to improved disinfection [McGuigan *et al.,* 1998]. Even extremely turbid water (200 NTU) can be disinfected under Kenyan sunlight after storing for 7 h at temperatures of 55 °C or higher [Joyce *et al.,* 1996]. An increase in dissolved oxygen in the PET bottles after agitation leads to improved inactivation [Kehoe *et al.,* 2000].

Figure 6 PET bottles filled with contaminated water and exposed to solar irradiation for water disinfection.

SODIS in PET bottles is limited to small volumes and sufficient supply of clean PET containers (**Figure 6**). Disinfection of larger volumes of water for various households with solar reactors is under study. Compound parabolic collector reactors (CPCs) are mostly used for this. Solar disinfection can be achieved even for real water sources in such reactors, but the effect has been noticeably improved by the presence of photocatalysts [Rincón and Pulgarin 2005 a Sichel *et al.,* 2007 b, c].

SODIS as a drinkingwater treatment has important advantages to alternative treatments i) availability in low-income, sun-rich areas ii) acceptance due to natural odor and taste of the water (often not the case for chlorine, [WHO, 2005]) iii) sustainability as no chemicals are consumed, and iv) no need of post treatment after disinfection. Following the standard

SODIS operating guidelines [WHO, 2005], solar inactivation should be sufficient to render potable water safe for drinking [McGuigan *et al.*, 2006; WHO, 2005].

These advantages led WHO to recommend SODIS for the reduction of health hazards related to drinking water on World Water Day on March 22, 2001. The acceptance of SODIS in many projects all over the world has been strongly supported by the Swiss Federal Institute for Environmental Science and Technology (EAWAG) whose scientists have been promoting SODIS for the last 4 years in more than 20 countries [EAWAG-SANDEC]. This already makes SODIS an important application for drinking water treatment, with even more potential for adoption in the future. Up to now there are no publications in the literature reporting the susceptibility of the *Fusarium* genus to solar disinfection in real water and under natural sunlight.

A new research project (FP6-2004-INCO-DEV-3-031650-SODISWATER) funded by the European Commission (VII FP), with the participation of the PSA and coordinated by the Royal College of Surgeons in Ireland (RCSI), is currently in progress to demonstrate that solar disinfection of real water can be an effective and acceptable intervention against waterborne disease for vulnerable communities in developing countries without reliable access to safe water, or in the immediate aftermath of natural or man-made disasters. One of the PSA tasks in this project is the development of a new solar photoreactor to disinfect water at house hold scale using different enhancements techniques to improve the efficiency of inactivation (e.g. continuous flow systems, CPCs, photocatalytic acceleration, etc.).

II. 13. Solar photocatalytic collectors

Concentrating photochemical systems: The first photo-reactors for solar photocatalytic applications designed at the end of the eighties were based on parabolic-trough collectors (PTC) [Pacheco and Tyner, 1990]. That was due, on one hand, to the historical emphasis that had been placed on this type of collectors for solar thermal applications, which made the technology relatively mature and easily adaptable [Kreider, 1979]. In the second place, among the concentrating, or better medium-concentrating, systems available, the PCTs were considered the most appropriate for this type of application. The parabolic-trough

collector consists of a structure that supports a reflective concentrating parabolic surface. The structure has a solar-tracking system that keeps the collector aperture plane perpendicular to the solar rays. In this situation, all the solar radiation available on the aperture plane is reflected and concentrated on the absorber tube that is located in the geometric focus of the parabola [Kelly and De Laquil, 1992].

At the end of the eighties and early nineties, two pre-industrial-size solar photocatalytic systems were designed and built at the National Solar Thermal Test Facility, located at the Sandia laboratories in Albuquerque, New Mexico (USA) [Pacheco *et al.*, 1993] and at the facilities of Plataforma Solar de Almería (Spain) [Minero *et al*., 1993]. Both facilities were based on parabolic-trough collectors with hundreds of square meters of collector surface and can be considered the starting point for solar photocatalytic technology development [Malato *et al*., 2004].

Non-concentrating photochemical systems: Non-concentrating solar collectors are stationary systems with no solar-tracking mechanisms, which are usually flat-plate, stationary devices oriented toward the equator at a specific inclination, depending on the latitude of the site. Their main advantage is their simplicity and lower manufacturing cost [Dillert *et al*., 1999]. Non-concentrating, stationary solar collectors are, in principle, more economical than PTCs, since there are no moving parts or tracking mechanisms.

However, they are less energy-efficient collecting beam sunlight, due to their fixed orientation with respect to incoming radiation. While they can make use of diffuse radiation, due to their wide "angle of acceptance" (see below), they are much more adapted to small-scale situations. Because of the great effort invested in small non-concentrating collector designs for other applications, a large number of non-concentrating solar reactors for solar photocatalytic processes have been developed and tested all over the world [Pacheco *et al*., 1993; Alfano *et al*., 2000; Wyness *et al*., 1994 a, b; Feitz *et al*., 2000; Franke and Franke, 1999] and particularly at the Plataforma Solar de Almería [Goslich *et al*., 1997; Guillard *et al*., 2003; Dillert *et al*., 1999; Malato *et al*., 2002 b, 2007, Malato *et al*., 2004].

Although non-concentrating flat reactors are more simple than concentrating systems, their design is not trivial, because of the requirements for chemical inertness and resistance both to weather and ultraviolet light. Furthermore, these systems require a significantly larger photoreactor surface for the same treatment volume than concentrating photoreactors and, therefore, must be designed to support high operating pressures in order to be able to pump the fluid. Photoreactors designed with any shape other than tubular may have serious disadvantages when scaling up to industrial scales. However tubular reactors are available in a large variety of materials and sizes and are a natural selection for any closed-flow system [Malato *et al*., 2004].

The main advantages of each of the different solar photocatalytic technologies can be summarized as follows [Goslich *et al*., 1997; Guillard *et al*., 2003; Dillert *et al*., 1999; Malato *et al*., 2002 b, 2007, Malato *et al*., 2004]:

Advantages of non-concentrating systems:

(i) they can make use of both direct and diffuse solar radiation; (ii) they are simpler, requiring a lower investment and less maintenance; (iii) water does not heat up significantly (iv) Quantum efficiency is better, since there is less recombination of electron/holes, given the lower photonic density than in concentrating systems.

Advantages of concentrating systems:

they have a noticeably smaller reactor tube area (a shorter loop in which to confine, control and handle the water); (ii) reactions that need high photone flux can be performed more easily.

II. 14. Compound Parabolic Collectors (CPC)

Compound Parabolic Collectors (CPCs) are used in thermal applications both with fins in non-evacuated concentrators and with tubes in vacuum collectors. For thermal applications, they are an attractive option, a cross between parabolic concentrators and stationary flat systems, since they combine the characteristics of both: they concentrate solar radiation, but like the flat plate collectors, they are stationary and collect diffuse radiation. Thus they are also a good option for solar photochemical applications.

CPCs have a reflective surface designed for ideal Non-Imaging Optics. They were invented in the 60s for solar concentration with stationary devices, and are able to concentrate all the radiation that arrives within the collector "angle of acceptance" onto the receiver. They do so by illuminating the complete perimeter of the receiver, rather than just the "front" of it, as in conventional flat plates glass [Blanco *et al*., 2000]. These concentrating devices have ideal optics even when designed for a concentration factor of 1 (CF=1). The concentration factor for a two-dimensional CPC collector is given by **Eq. 6**.

$$CF_{CPC} = \frac{1}{\sin \theta_a} = \frac{a}{2\pi r} \qquad \textbf{(Eq. 6)}$$

Where (θ_a) is the normal semi-angle of acceptance, *r* is the absorber radius and *a* is the aperture area of the collector.

The normal semi-angle of acceptance (θ_a), for photocatalytic applications is between 60 and 90 degrees. This wide angle of acceptance allows the receiver to collect both direct and a large part of the diffuse light, with the additional advantage of decreasing errors of both the reflective surface and receiver tube alignment, which become significant for a low cost photoreactor. When θ_a = 90º, whereby CF = 1 (non-concentrating solar system) and each CPC curve is an ordinary involute, all the UV radiation that reaches the aperture area of the CPC (not only direct) can be collected and redirected to the reactor. By designing the CPC for an acceptance angle of +90º to -90º, all incident solar diffuse radiation can be collected. The light reflected by the CPC is distributed all around the tubular receiver so that almost the entire circumference of the receiver tube is illuminated and the light incident on the photoreactor is the same that would be impinging on a flat plate (**Figure 7**).

The advantages of CPCs can be summarized as follows. Just as in the parabolic-trough collector, in CPC-type collectors, the photoreactor is tubular so the water can be pumped easily. CPC devices for photocatalytic applications are generally fabricated with aluminum reflectors and the structure is usually a simple frame that, in turn, is the support for connecting the photoreactor tubes, normally glass [Blanco *et al*., 2000].

Solar CPC reactors for water detoxification: According to a review by Goswami [Goswami, 1997], until the late 1990s only two engineering-scale demonstrations plants for AOP processes with CPCs had been reported: one for groundwater treatment in the USA, and one for industrial wastewater treatment in Spain. Then more and more pilot plants emerged, most of them using the photo-Fenton process or TiO_2 photocatalysis. Dillert *et al*. installed a pilot plant at the Volkswagen AG factories in Wolfsburg (Germany) to decontaminate biologically pre-treated industrial wastewater using the Double Skin Sheet Reactor (DSSR) [Dillert *et al*., 1999]. The flowchart of a more recent version of this pilot plant, which was installed in 2000, has recently been published [Bahnemann, 2004]. In 1997, Freudenhammer *et al*. reported their results in a pilot study with the TFFBR (Thin Film Fixed Bed Reactor) in various Mediterranean countries [Freudenhammer *et al*., 1997; Geissen *et al*., 2001]. Based on these results, a pilot plant, financed by the European Commission, has been built at the site of a textile factory in Tunisia (Menzel Temime) [Bahnemann, 2004; Bousselmi *et al*., 2004].

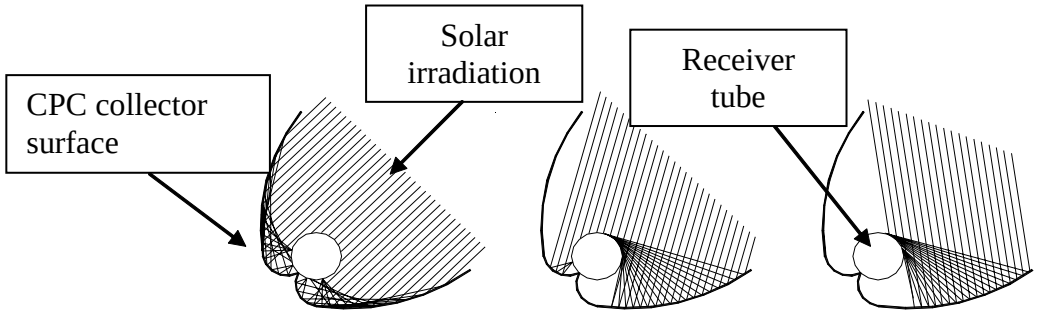


Figure 7 Diagram of reflected radiation on the CPC mirror surface [after Blanco *et al*., 2000]

Under the "SOLARDETOX" project (Solar Detoxification Technology for the Treatment of Industrial Non-Biodegradable Persistent Chlorinated Water Contaminants), a European consortium was formed for the development and marketing of solar detoxification of recalcitrant water contaminants (financed by the EC-DGXII through the Brite Euram III Program, 1997–2000) [Blanco *et al*., 2000]. The project focused on the development of a commercial non-concentrating solar detoxification system using the compound parabolic collector technology (CPC), with a concentration ratio = 1. A full-size field demonstration plant was erected at the facilities of HIDROCEN (Madrid, Spain) (**Figure 8**).

This solar photocatalytic plant was designed with 100 m^2 of collector aperture area designed in industrial-scale. It was the one of the first projects demonstrating that the solar photocatalytic technology is sufficiently developed for industrial use [Malato *et al.*, 2002 a]. Even recycling the catalyst was included in the treatment plant [Fernández-Ibáñez *et al*., 2003].

Similar work with CPC reactors has been reported for treatment of paper mill water effluents in Brazil and Germany [Sattler *et al*., 2004; Machado *et al*., 2003 and 2004], and surfactants [Amat *et al*., 2004] in Spain.

Figure 8 Partial view of the demonstration plant based on CPCs [Malato *et al*., 2002 a]

More recently (2004), a new CPC plant, has been installed in a project focusing on problems in the rapidly growing intensive greenhouse agriculture sector in the Mediterranean Basin for the treatment of pesticide contaminated water. The plant is now in routine operation [Blanco-Gálvez *et al.*, 2007].

Solar CPC reactors for water disinfection: Vidal *et al.* (1999, 2000) published the first pilot plant study of TiO_2 solar photocatalysis for water disinfection. This team built a low-cost CPC prototype with 4.5-m^2 CPC. The results for *E. coli* and *Enterococcus faecalis*

(initial concentration ~ 10^2-10^5 CFU/mL), with TiO_2 suspensions (0.5 g/L), showed a 5-log decrease after 30 min of solar irradiation (where the solar UV averaged around 25 W/m^2).

Recent work has studied improvement of solar disinfection using supported TiO_2 on resistant flexible materials, like cylinders, beads, balls, mesh, etc. One example is TiO_2 deposited on fiberglass [Ahlstrom, 1999] inserted in a tubular photoreactor in a CPC solar collector [Fernández *et al.*, 2005]. The authors demonstrated that the CPC solar photoreactor is efficient for *E. coli* inactivation by solar photocatalysis with TiO_2 slurries and supported TiO_2 with treatment periods of 30-60 min.

McLoughlin *et al.* (2004) studied the use of three types of static solar collectors for the disinfection of water containing *E. coli*. They demonstrated that three lab-scale solar photo-reactors with aluminum reflectors consisting of compound parabolic, parabolic and V-groove profiles all enhance the effect of the natural solar radiation, although the CPC is more efficient than the parabolic or V-groove profiles. They also proved that low concentrations of titanium dioxide on a rod inserted in the reactors moderately enhance the overall disinfection performance in the CPC [McLoughlin *et al.*, 2004 a, b].

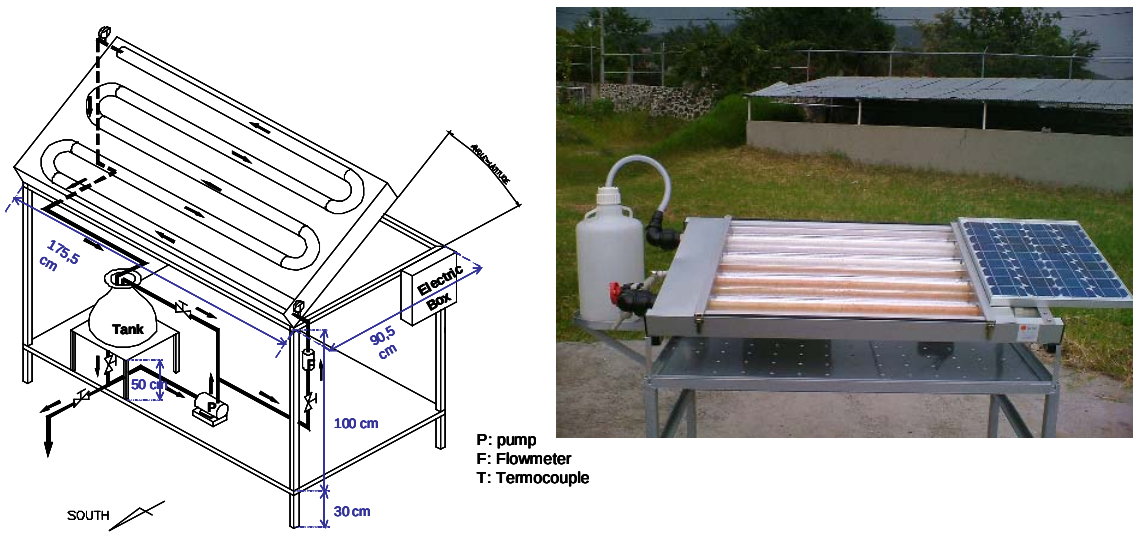


Figure 9 Scheme and picture of the in AQUACAT and SOLWATER projects developed photoreactor for photocatalytic drinking water disinfection in developing countries [Malato *et al.* 2007].

Very recent research work for concrete applications in the field of solar photocatalytic water treatment has also been sponsored by the European Commission's International Cooperation (INCO) Program in two different projects. Both projects aimed at developing a cost effective technology based on solar photocatalysis for water decontamination and disinfection in rural areas of developing countries (SOLWATER Project: http://www.psa.es/webeng/solwater/index.html; AQUACAT Project: http://aquacat.univ-lyon1.fr/). As a final research step, both projects developed solar reactors to decontaminate and disinfect small volumes of water (**Figure 9**).

The target was drinking water treatment in households and small communities and the research work was carried out with *E. coli* and humic acids [Sichel *et al.* 2005]. Also within these projects, real field tests were carried out with these prototype photoreactors installed in Argentina, Egypt, Mexico, Morocco, Peru, and Tunisia [Malato *et al*., 2007]. In 2007, results published reported the successful operation of the prototypes under real conditions [Navntoft *et al*., 2007].

III. Goals of this thesis

- Develop a solar photocatalytic disinfection method with TiO_2 able to disinfect fungi of the *Fusarium* genus.

- Find out the response and resistance of different *Fusarium* species and spores to the solar photocatalytic disinfection treatment.

- Scale up an operable TiO_2 disinfection method from laboratory scale to the disinfection of various liters in solar CPC reactors. Test of the effects of experimental conditions on the photocatalytic disinfection of different *E. coli* test organisms.

- Find out the influence of bare solar UV irradiation, UV- intensity and dose on microorganisms in water and on photocatalytic disinfection with TiO_2 for bacteria and fungi.

- Test an alternative disinfection method, the synergistic action of solar UV-radiation and H_2O_2. Prove that this method causes lethality of fungal spores in distilled or natural water at laboratory and Solar CPC reactor scale, to demonstrate its application potential.

- Achieve TiO_2 disinfection in solar CPC reactors for *Fusarium* in real water sources to ensure its potential application to agricultural irrigation water disinfection.

- Design a complete pilot plant scale disinfection process with pre and post treatment units.

IV. 1. Solar photocatalytic disinfection of agricultural pathogenic fungi: *Fusarium* species

Abstract

The ability of solar-only and solar photocatalytic (TiO_2) disinfection batch-process reactors to inactivate fungal pathogens was evaluated. The photocatalytic disinfection of five wild strains of the *Fusarium* genus (*F. equiseti, F. oxysporum F. anthophilum, F. verticillioides*, and *F. solani*), a common plant pathogen in Spain and around the world, was successfully achieved. Different disinfection times (1 to 6 hours) were necessary to inactivate a fungus concentration in water of 10^3 CFU/mL to almost zero by solar photocatalysis. The order of sensitivity to solar disinfection was *F. oxysporum* > *F. solani* > *F. verticillioides* > *F. anthophilum* > *F. equiseti* . The presence of the TiO_2 photocatalyst under solar radiation showed a positive effect on lost fungus viability. The photocatalytic disinfection times were shorter and disinfection better than for solar-only disinfection. The order of photocatalytic sensitivity was different from solar disinfection: *F. verticillioides* > *F. oxysporum* > *F. solani* > *F. anthophilum* > *F. equiseti*.

Keywords: Solar disinfection, TiO_2 photocatalysis, *Fusarium*, hydroponics, soilborne fungus.

Introduction

The amount of land devoted to hydroponic agriculture increases every year in certain areas of the world, and large commercial greenhouse operations are also expanding in semiarid areas with high solar radiation and sufficient underground water. These soilless growing systems employ organic or inorganic inert substrates based on perlite [Vázquez, 2005], rockwool [Bussell, 2004; Le Bot, 2006] or coconut fiber [Raviv, 2005] and liquid nutrient solutions, creating a pathosystem environment in the plant root zone that is very different from traditional agriculture.

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The transition from soil to soilless culture has not resulted in the disappearance of soil-borne diseases, but in the appearance of water-borne diseases [Runia, 1994]. Recirculation of the nutrient solution in such cultures has obvious environmental and economic advantages: saving water and fertilizer, high-density planting, maximum crop yield, crop production. Nevertheless, problems arising from the accumulation of phytopathogens are far from being solved. Various chemical fungicides (ethridiazol, furalaxyl, methalaxyl, benomyl, copper oxalate and oxyquinoline sulfate) tested for pathogen control have often turned out to be phytotoxic in soilless cultures. Results with chlorine have been disparate and often phytotoxic. Surfactants and sodium hypochlorite have demonstrated limited success in controlling *Olpidium bornovanus* [Tomlinson and Thomas, 1986].

Innovative, efficient methods for achieving optimum control in such horticultural systems necessarily involve efficient disinfection of the nutrient solution and precise in-situ monitoring of the main species. Therefore, developed countries such as the Netherlands or Sweden are already investigating methods of achieving their optimum control [Runia, 2001], for example, used UV disinfection lamps (254 nm) to treat water containing *Phytium* and *Phytophtora*. The vast majority of pathogens relevant to soilless cultures belong to the order of oomycetes which are characterized by their asexual free-swimming spores called zoospores. This particular feature makes them a high risk to a recirculating water culturing medium [Jung, 2003, Gomez Vázquez, 2003].

In this regard, it is essential to evaluate different alternatives to ensure adequate control of such pathogens. These alternatives should focus on low production costs and be environmentally friendly. Advanced oxidation processes (AOPs) may be used for decontamination of water containing hazardous organic compounds [Pera-Titus *et al.,* 2004]. Although there are different reaction systems, all of them are characterized by the production of hydroxyl radicals ($^{\bullet}OH$), which are able to oxidize and mineralize almost any organic molecule, yielding CO_2 and inorganic ions. Heterogeneous photocatalysis is based on the use of a wide-band-gap semiconductor (e.g. TiO_2), in which photo excitement of a valence band electron leads to several reactions and production of the desired hydroxyl radical [Devipriya *et al.,* 2005]. TiO_2 photocatalysis has been proven effective not only for degradation of organic pollutants [Augugliaro *et al.,* 2004; Kositzi *et al.,* 2004;

Wiszniowski *et al.,* 2004], but for disinfection of bacteria, viruses, cancer cells, yeast, etc. in water [Blake *et al.,* 1999]. Several solar TiO_2 photocatalytic applications have also been demonstrated to be feasible for water disinfection purposes [Fernández *et al.,* 2005; Malato *et al.,* 2007]. In this regard, the well-known process called "solar water disinfection" or SODIS, is very important, because the solar radiation, apart from the effect of mere exposure, inactivates microorganisms present in the water by thermal effects (above 45 ºC) [McGuigan *et al.,* 1998]. Solar disinfection is mainly attributable to the radicals produced by the energy in the solar spectrum [Gelover *et al*., 2006; Rincón and Pulgarin 2004]. The SODIS method has been proven successful in the disinfection of a wide range of microorganisms [Joyce *et al.,* 1996; McGuigan *et al.,* 2006]. However, not many contributions work on photocatalytic disinfection of fungi [Wolfrum *et al.,* 2002; Seven *et al.,* 2004; Lonnen *et al.,* 2005; Maneerat and Hayata, 2006].

Lonnen and coworkers demonstrate that the fungal pathogens *C. albicans* and *F. solani* are readily inactivated using batch-process photocatalysis and solar disinfection under simulated solar radiation [Lonnen *et al.,* 2005]. The TiO_2 photocatalytic reaction in the form of TiO_2 powder and TiO_2 coated on a plastic film was found by Maneerat and Hayata to have antifungal activity on *Penicillium expansum*. These authors suggest after their research with in vitro and fruit tests that this treatment has potential for post-harvest disease control [Maneerat and Hayata, 2006]. Seven *et al.,* (2004) investigated the photocatalytic inactivation of, among other microorganisms, two types of fungus: *Aspergillius niger* and *C. albicans*. They report the results of photocatalytic water disinfection using TiO_2 and ZnO as photocatalysts and a sodium lamp as the source of photons. Those authors demonstrated the complete inactivation of *C. albicans* with both photocatalysts, however, they were not successful for *A. niger* [Seven *et al.,* 2004].

The purpose of this paper is to investigate photocatalytic fungal inactivation in water with natural solar radiation as the source of light. The fungi used in this work are wild strains of the *Fusarium* genus. *F. solani* and the different *formae speciales* of *F. oxysporum* are well-known soilborne pathogenic fungi of horticultural crops. *F. equiseti*, *F. anthophilum* and *F. verticillioides* have a less-known pathogenicity, but in our own experiments they all have shown a certain pathogenicity in seedlings of tomato and melon when were cultivated in

vermiculite (a soiless substrate) [Tello and de Cara, unpublished data]. The various *Fusarium* species develop various types of spores: microconidia, macroconidia and chlamydospores. The main difference between the different spores is the cell wall. Microconidia and macroconidia have a single wall and they don't survive when adverse conditions are present. Otherwise, chlamydospores have a double wall and they have been always considered the conservation structures of the fungi [Bartual *et al.,* 1993]. These worldwide pathogenic species are commonly found in agriculture. Their control is especially important in areas where intensive hydroponic agriculture is taking hold, as in Europe, North America, Middle East and Australia [Nelson *et al.,* 1994]. In this paper, inactivation in water of the five fungi mentioned is studied with and without the addition of TiO_2. Particular emphasis is placed on analysis of the solar photocatalytic process rate.

Materials and Methods

Fungal strains preparation & sampling: The fungi used were *F. equiseti, F. oxysporum F. anthophilum, F. verticillioides,* and *F. solani.* The strains were original wild fungi obtained from soil cultures in Almería (southern Spain). The *Fusarium* fungi colonies were transferred to the sporulation medium: agar containing sodium and potassium chloride [Tello *et al.,* 1991] in Petri dishes exposed to UV-C radiation from a mercury lamp (40 W) for 15 days at 25 ºC to produce the required number of spores (10^5-10^7 CFU/plate, depending on the *Fusarium* species). The spores were detached from the mycelium and from the agar by washing the plates with distilled water. The suspension obtained was homogenized by mechanical agitation and the concentration was determined by direct count using a phase contrast microscope (Section 2.5). The suspension was diluted into the bottle-reactors to obtain an initial spore concentration of ca. 10^3 CFU/mL.

50 μL samples of the water were plated out using the spread-plate technique on acidified malt agar (Sigma Aldrich, USA), (pH= 4). Where fewer than 10 colonies per plate were observed, 500 μl of the sample were plated for a detection limit of 2 CFU/mL. Each measurement was made in triplicate, with three sample inoculations on three plates. After two days of incubation at 28 °C in the dark, the colonies were counted.

Solar experiments: The experiments were performed in 250-mL DURAN-Glass (Schott, Germany) batch reactor bottles. Glass covers (Shott) were used to allow the solar radiation to enter the bottle-reactor from all directions. The bottles were stirred with magnetic stirrers at 100 rpm during the experiment. All experiments were performed at the Plataforma Solar of Almeria (latitude: 37.0909 ºN, longitude: 2.357 °W), under natural sunlight in triplicate. The solar photocatalytic and solar disinfection tests were carried out simultaneously and the control bottles (one with and another without TiO_2) were stored under the same conditions, but in the dark for each *Fusarium* species. The results of every three replicates were highly reproducible. The experimental error is around 10% when the concentration of fungus was over 50 CFU/mL, between 50 and 2 CFU/mL the error obviously increased. These percentages are normal for the plate count technique. Each point shown in the graphs represents the average of triplicates, and the error bars correspond to the statistical error of the triplicates at 95% confidence level.

The temperature, measured during all experiments, was always below 35 °C, allowing thermal inactivation of fungi to be discarded. Nevertheless, the control samples in the dark were kept at the same temperature as solar experiments, to avoid thermal differences between irradiated and non-irradiated samples. All solar experiments started 3 hours before noon and finished 3 hours after noon so they were performed under similar irradiation conditions.

The TiO_2 P25 (Degussa, Germany) catalyst was used as a slurry. The optimal catalyst concentration for this kind of experimental set up was estimated with an equation published before for solar reactors [Fernández-Ibáñez *et al.,* 1999]. This concentration was calculated to be 35 mg/L, at which the total light extinction is 99% of the incoming solar intensity.

UV-A solar radiation measurement & assessment: Time-dependent results for each *Fusarium* species were evaluated to compare solar photocatalytic and solar inactivation, and dark control samples during simultaneous experiments. A solar energy unit, Q_{UV}, is employed to take the different radiation conditions (solar experiments with different fungus strains) into account. The Q_{UV} value estimates the accumulated UV-A energy in the

photoreactor per unit of volume of treated water for a certain period of time during the experiment. Thus Q_{UV} standardizes the energy dose in the photocatalytic reaction under natural sunlight. Solar UV-A radiation intensity was measured at the Plataforma Solar by a global UV-A radiometer (Mod. CUV3, KIPP&ZONEN, the Netherlands) with a typical sensitivity of 264 μV/W per m^2, which provides data in terms of incident W_{UV}/m^2. This can then be used in Equation 2 to calculate the total UV-A energy received per unit of volume, Q_{UV} [Fernandez *et al.,* 2005], where t_n is the experimental time for n-sample, $\overline{UV}_{n-1}$ is the average solar ultraviolet radiation measured during the period $(t_n\text{-}t_{n-1})$, A_r is the illuminated surface, and V_t the total water volume.

$$Q_{UV} = \sum_n \overline{UV}_{n-1} \frac{A_r}{V_t}\left(t_n - t_{n-1}\right) \qquad \textbf{(Eq. 1.1)}$$

Optical Phase-Contrast and Clearfield Microscopy: A Nikon, Mod. Eclipse 50i (Japan) optical microscope was used for counting microorganisms and TiO_2 adsorption monitoring. Pictures taken during adsorption were captured in clear field mode with a Nikon Coolpix 8400 camera. 50 mg/mL malachite green was used as a stain. Phase contrast mode was used for Neubauer counting.

Results

Disinfection of F. equiseti: F. equiseti is the *Fusarium* species currently receiving most of the attention worldwide from researchers working with soil-borne fungi because it is so common. Although the phytotoxicity of this fungus has not yet been proven, it was studied as a model fungus that produces two target spores, macroconidia and chlamydospores. Fungal inactivation by sunlight occurs with and without addition of TiO_2 (**Figure 1.1**). However, direct fungicidal action of sunlight with the addition of TiO_2 as shown in **Figure 1.1** is much more effective than the action of solar radiation alone. In fact, while the TiO_2 photocatalytic experiments show a 3-log decrease, the blank tests only show a decrease of almost 0.5 log. The total abatement of *F. equiseti* is only achieved in the presence of TiO_2, whereas solar exposure alone only has a very slight effect on fungus survival. This agrees with previous results for fungi and other types of microorganisms, such as bacteria [Rincón and Pulgarin, 2001; Fernández *et al.,* 2005], fungi [Lonnen *et al.,*

2006; Seven *et al.,* 2004], virus and cancer cells [Blake *et al.,* 1999]. In the dark, for 6 hours with constant agitation at 25 ºC with and without addition of TiO_2, all *F. equiseti* colonies survive; this behaviour is coherent with the studies on bacteria [Matsunaga and Okochi, 1995; Rincón and Pulgarin 2001], which demonstrate that titanium dioxide has no significant effect on survival of bacteria in the dark.

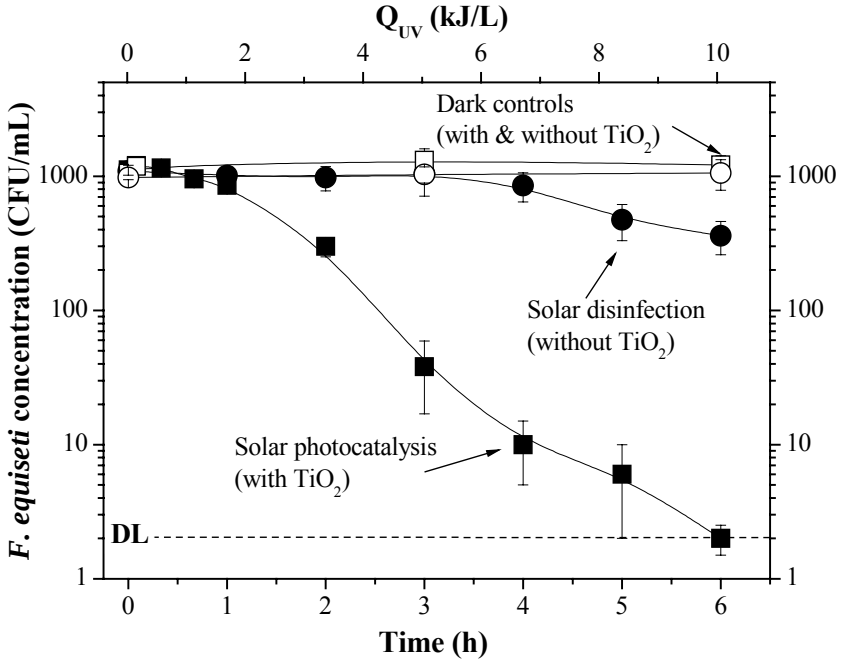

Figure 1.1 Effect of TiO_2 (35 mg/L) photocatalysis on *F. equiseti* survival. Initial concentration 1.2 x 10^3 CFU/mL. Inactivation of *F. equiseti* with (–■–) and without addition of TiO_2 (–●–) under natural solar radiation, and dark controls (–□–, –○–). Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

As both series of experiments (solar with and without TiO_2) were performed simultaneously under perfectly sunny conditions, comparison over Q_{UV} (top axis in **Figure 1.1**) shows the same disinfection kinetics. It can be observed that 6 hours of natural solar irradiation were necessary to inactivate *F. equiseti* spores from 1200 to 2 CFU/mL (detection limit). During this period, the total UV-A energy received per unit of volume was 10.05 kJ/L, and the average solar UV-A irradiance was 25.78 W/m^2.

These experiments were closely monitored by measurement of other parameters like pH of water, average UV-A irradiance at different times, temperature and conductivity. The highest temperature was 25 °C, which is below the optimal growth temperature of

F. equiseti. The solar UV-A intensity changed over time. During the first hour, average UV-A irradiance was low (between 15 and 22 W/m^2), and the concentration of *F. equiseti* did not diminish with either solar radiation or solar photocatalysis. However, from 1 to 4 h the UV-A irradiance increased from 22 to 34 W/m^2 and photocatalytic inactivation of *F. equiseti* provoked a 2-log decrease, while solar exposure alone still did not induce any change in *F. equiseti* concentration. The tendency to inactivation remained the same during the last two hours in both processes. In view of these results, it may be said that solar photocatalytic inactivation of this fungus is noticeably influenced by solar UV-A irradiance, mainly when UV-A irradiance is over 20 W/m^2. This very wide difference in behaviour of *F. equiseti* with solar exposure alone and solar photocatalysis was not observed for all the *Fusarium* species we studied.

The other fungi strains are more sensitive than *F. equiseti* to both treatments, solar and solar photocatalytic disinfection, as shown below. During photocatalytic disinfection, the pH dropped from 6 to 4, whereas pH remains constant in the tests made without addition of TiO_2. This drop was not observed in any of the photocatalytic reactions with the other *Fusarium* species and can be attributed to the compounds generated during photocatalysis, since it is not due to TiO_2 reaction with water [Kosmulski, 2002]. Conductivity remained between 240 and 260 $\mu S \cdot cm^{-1}$, which is high compared to distilled water. This is due to the ions that entered the water during the spore preparation protocol.

Disinfection of F. oxysporum: F. oxysporum is a very common plant pathogen in many types of crops, producing macroconidias, microconidias, and chlamydospores under UV-A light. **Figure 1.2** shows the evolution of this fungus with solar-only and solar photocatalytic disinfection. It has been shown that *F. oxysporum* can be disinfected photocatalytically and by solar irradiation alone. The detection limit for TiO_2 photocatalysis was already reached by *F. oxysporum* after four hours of photocatalysis, whereas for *F. equiseti* complete disinfection took 6 h. Complete solar-only disinfection required 5 h of solar irradiation. After a three-hour delay, solar disinfection started to reduce the concentration noticeably, but did not reach a disinfection rate comparable to TiO_2 disinfection.

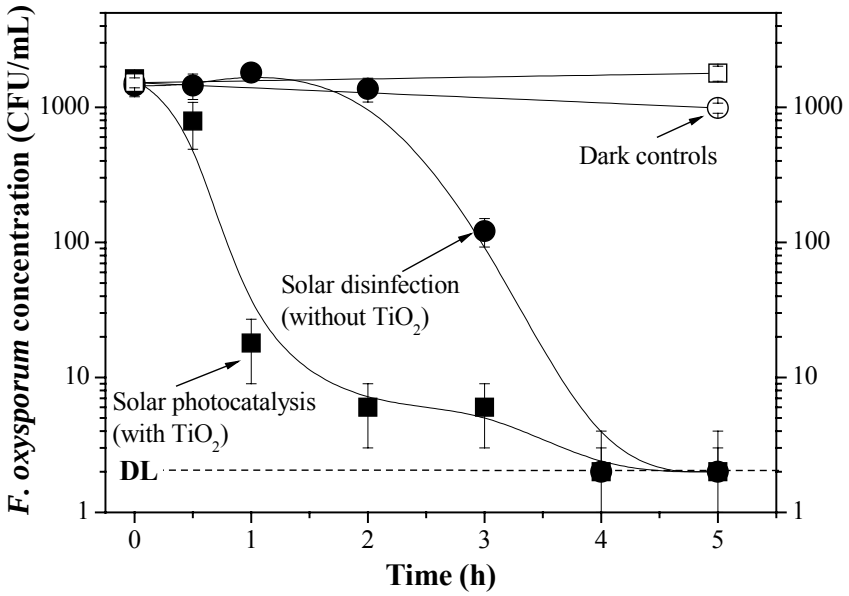

Figure 1.2 Inactivation of *F. oxysporum* with (–■–) and without addition of TiO_2 (–●–) under natural solar radiation, and dark control samples (–□–, –○–). Initial concentration 6 x 10^3 CFU/mL. Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

Disinfection of F. anthophilum: *F. anthophilum* is a common agricultural pathogen which produces microconidas and macroconidias. **Figure 1.3** shows the evolution of *F. anthophilum* concentration during solar-only and solar photocatalytic disinfection. While TiO_2 disinfection reached the detection limit after 3 hours, solar disinfection was much slower, and was still not complete after 5 hours of solar irradiation.

Disinfection of F. verticillioides: *F. verticillioides* is another common pathogen of the *Fusarium* genus. It produces microconidias only when exposed to UV-A light. **Figure 1.4** shows the experimental series performed under solar radiation during 6 hours with and without TiO_2. With TiO_2 disinfection, the detection limit was reached within the first hour of treatment, and with solar-only disinfection, after 5 h. It can be observed that this fungus, which only produces microconidias, was much more sensitive to TiO_2 disinfection than the other species in the series, while its sensitivity natural solar radiation is not very high and is similar to the other species studied.

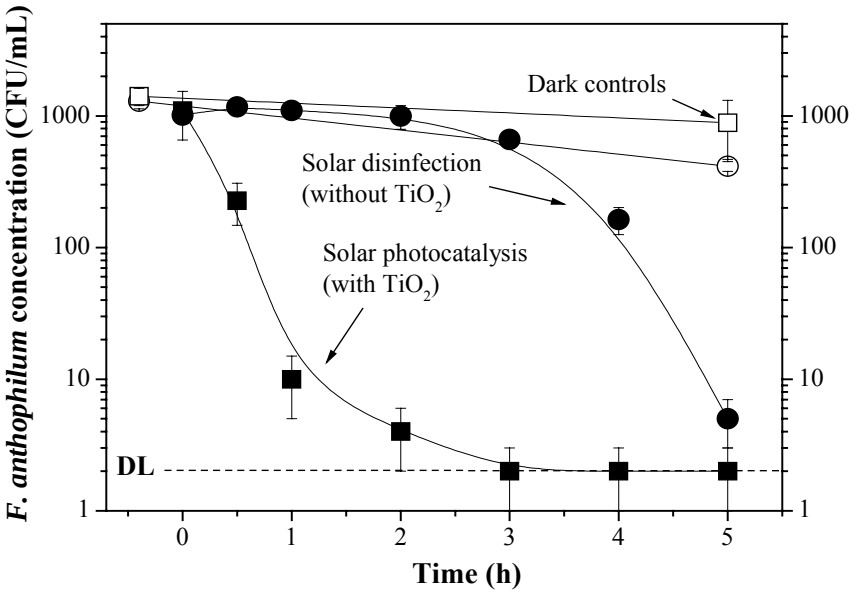

Figure 1.3 Inactivation of *F. anthophilum* with (–■–) and without addition of TiO_2 (–●–) under natural solar radiation, and dark control samples (–□–, –○–). Initial concentration 1400 CFU/mL. Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

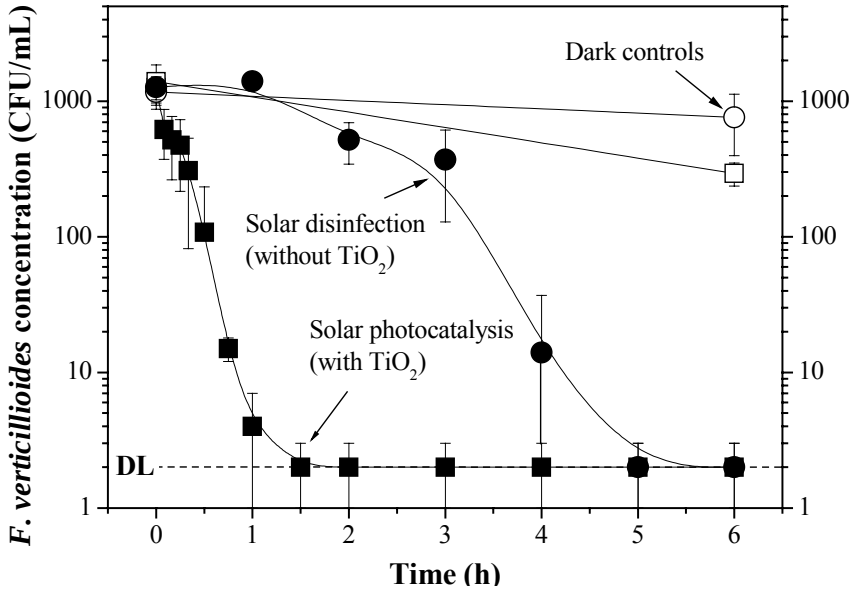

Figure 1.4 Inactivation of *F. verticillioides* with (–■–) and without (–●–) addition of TiO_2 under natural solar radiation, and dark control samples (–□–, –○–). Initial concentration 1200 CFU/mL. Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

Disinfection of F. solani: F. solani produces microconidia, macroconidia and chlamydospores. **Figure 1.5** shows the evolution of the concentration of *F. solani* during solar-only and solar photocatalytic disinfection. Both processes led to total disinfection

after 4 h, although the inactivation kinetics show noticeable differences. While TiO_2 disinfection decreases the concentration one log within the first hour, solar disinfection only provokes a very slight decrease during the first 2 hours of the experiment. After this delay, solar-only disinfection reaches an inactivation rate comparable to TiO_2 disinfection.

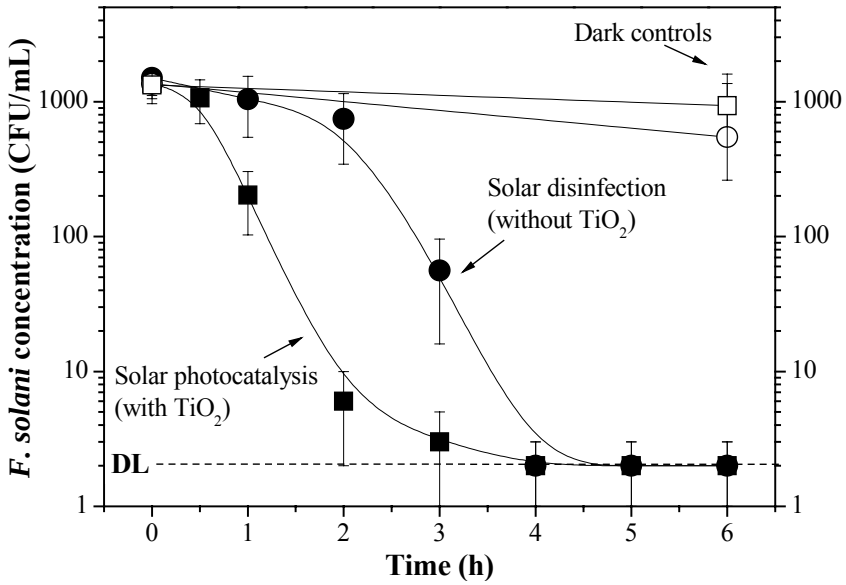

Figure 1.5 Inactivation of *F. solani* with (–■–) and without addition of TiO_2 (–●–) under natural solar radiation, and dark control samples (–□–, –○–). Initial concentration 1300 CFU/mL. Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

Comparison of results

Solar disinfection results for all the *Fusarium* species studied in this work can be compared with the solar UV-A energy per unit of volume (Q_{UV}, kJ/L) as shown in **Figure 1.6**. The response of all *Fusarium* sp. studied to global solar energy was analysed during solar-only and photocatalytic experiments (data not shown). It was found very similar in all cases. Therefore, it may be assumed that global solar energy is not a good parameter for evaluating the process efficiency, due ton the fact that the higher energetic UV-A part of the solar spectrum is mainly responsible for disinfection.

Disinfection of the *Fusarium* sp. studied by sunlight was observed to be different in the absence and presence of TiO_2. In the absence of TiO_2, fungus inactivation during illumination is very slow at first. All solar-only results show a shoulder effect followed by fast inactivation at an inactivation rate similar to photocatalysis. This solar disinfection

shoulder effect has previously been reported for *E. coli* [Pulgarin and Rincón, 2003; Berney *et al.,* 2006 a, 2006 b].

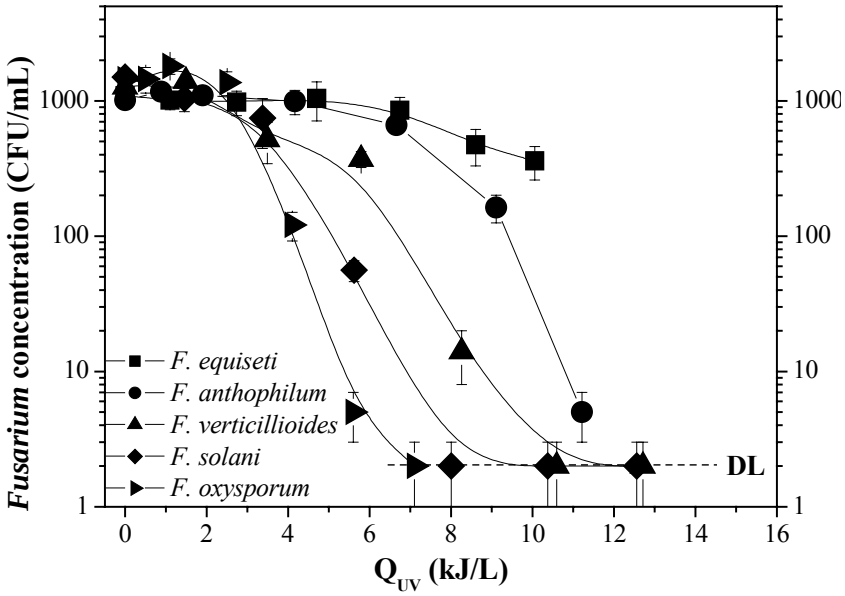

Figure 1.6 Solar disinfection of the *Fusarium species* studied without addition of TiO_2 under natural solar radiation plotted against Q_{UV}. Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

As suggested by those authors, in our case, this effect can be explained by the self-defence mechanisms induced by UV-A in the injured spores, which weakens the effect of UV-A light at the beginning of solar irradiation. On the other hand, in the presence of TiO_2, the large number of $^{\bullet}OH$ radicals produced, immediately break down spore self-protection mechanisms. Toward the end of phototreatment, inactivation of spores is slower because the remaining resistant spores in the irradiated water compete for $^{\bullet}OH$ with both the inactivated spores and the metabolites released during the photoprocess. The differences in these inactivation mechanisms may explain the different sensitivities of the fungus spores studied to solaronly and solar TiO_2 photocatalysis. Studies on the photocatalytic inactivation of bacteria suggest that oxidative damage first takes place on the cell wall, where the TiO_2 photocatalytic surface first makes contact with whole cells [Huang *et al.,* 2000, Kiwi and Nadtochenko, 2005].

It is unclear what reactive oxygen species are directly involved in the photokilling process, especially the identities of the main reactive oxygen species (ROS), which not only include the OH radical, but also $O_2^{\bullet-}$, and H_2O_2 [Sunada *et al.,* 2003]. Cho *et al.,* demonstrated the important role of the $^{\bullet}OH$ in the inactivation of *E. coli* in presence of UV-illuminated TiO_2 [Cho *et al.,* 2004].

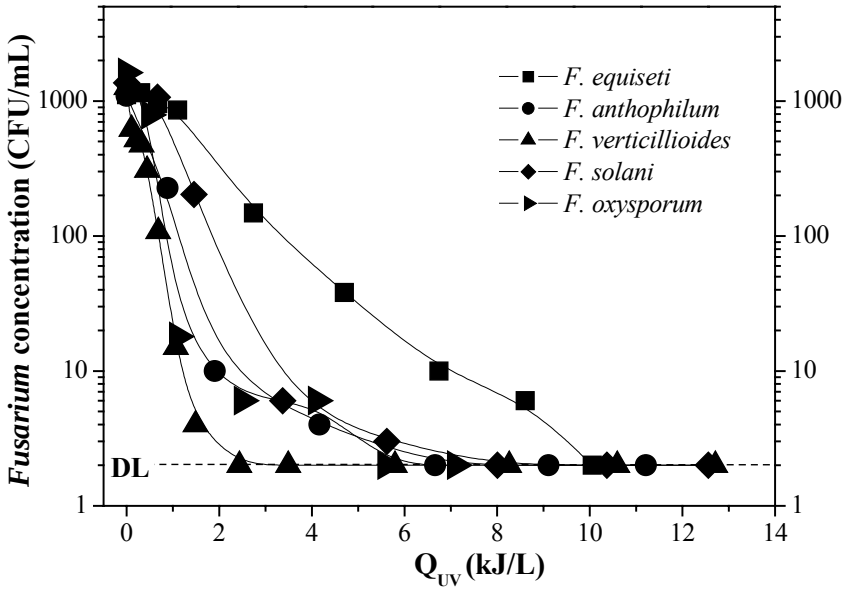

Figure 1.7 Solar disinfection of the *Fusarium species* studied with TiO_2 solar photocatalysis versus Q_{UV}. Each point represents the average of triplicates, and bars show the statistical error at 95% confidence level.

F. equiseti becomes the species most resistant to solar irradiation due to the formation of spores (macroconidia and chlamydospores) which are more robust than those formed by the other fungi. In fact, *F. solani* and *F. oxysporum* also produce macroconidias but very few chlamydospores, while *F. verticillioides* and *F. anthophilum* only generate microconidias and very few macroconidias. Microconidias are smaller than macroconidias, but chlamydospores are the most resistant because they have a double cell membrane while the other conidia have a single cell wall [Nelson *et al.,* 1994].

This order of resistance is slightly different in solar photocatalysis in **Figure** 1.**7**, where *F. verticillioides* and *F. oxysporum* are seen to be the most easily inactivated fungi followed by *F. anthophilum* and *F. solani* and by *F. equiseti*, which is the hardest to inactivate even in the presence of TiO_2.

Table 1.1 Solar photocatalytic inactivation over UV-A radiation for a variety of fungi.

Solar photocatalysis vs. UV-A radiation					
Fungus species	Q_{UV} (kJ/L)	UV-A dose (J/m^2)	Inactivation (log)	Efficiency factor: Inactivation/Q_{UV} (L/kJ)	Resistance
F. equiseti	8.60	0.521	2.3	0.27	+++
F. anthophilum	4.20	0.255	2.4	0.57	+
F. solani	3.36	0.204	2.5	0.74	-
F. oxysporum	6.50	0.390	2.9	0.45	--
F. verticillioides	1.50	0.091	2.5	1.67	---

To compare the disinfection efficiency of different fungi, an efficiency factor is defined as the efficiency factor of inactivation (in terms of logs decrease) to solar energy (UV-A) received per unit of volume. Therefore, the lower the efficiency factor the more resistant the species and vice versa. **Table 1.1** shows the inactivation: Q_{UV} efficiency factor in the photocatalytic experiments. It may be observed that *F. verticillioides* is inactivated six times faster than *F. equiseti*. The order of resistance to photocatalysis also shown in this table is coherent with the robustness of the spores produced by each fungus.

Table 1.2 Solar inactivation over UV-A radiation for a variety of fungi.

Solar inactivation vs. UV-A radiation					
Fungus species	Q_{UV} (kJ/L)	UV-A dose (J/m^2)	Inactivation (log)	Efficiency factor: Inactivation/Q_{UV} (L/kJ)	Resistance
F. equiseti	10.1	0.612	0.5	0.05	+++
F. anthophilum	11.2	0.679	2.3	0.21	+
F. verticillioides	10.6	0.642	2.8	0.26	-
F. solani	8.0	0.485	2.9	0.36	--
F. oxysporum	7.1	0.426	2.9	0.41	---

Table 1.2 shows the "inactivation: Q_{UV}" efficiency factor for solar disinfection. In view of these values, it can be deduced than the order of resistance remains almost the same as for photocatalysis, except for *F. solani, F. oxysporum,* and *F. verticillioides*. This fact could be attributed to the different sensitivities of the fungi to the disinfection processes and to the fungi having developed a different photo-repair response as a defence mechanism.

As with chemical methods, the performance of UV disinfection systems is usually based on the use of UV lamps (254 nm) and is characterized by the disinfecting UV dose. The most common way to compare the resistance of various microorganisms to a UV-A disinfection system is to determine the "UV-A dose" in terms of kJ/m^2, defined as the product of UV-A intensity and irradiation time. In this work the solar UV-A dose is given in **Tables 1.1** and **1.2.**

Our results are in agreement with previous work by researchers using various other types of microorganisms, like *E. coli, Lactobacillus acidophilus, Streptococci,* etc. [Matsunaga and Okochi, 1995; Blake *et al.,* 1999; Wist *et al.,* 2002; Rincón and Pulgarin, 2004]. A few recent studies have demonstrated the ability of TiO_2 photocatalysis to inactivate fungi. Seven *et al.* disinfected various microorganisms, with an initial concentration of around 10^5 CFU/mL, in a short period of time by using a 400 W sodium lamp in the presence of TiO_2 and ZnO photocatalysts (1 mg/mL) [Seven *et al.,* 2004]. In particular, *C. albicans* was inactivated by the irradiation of TiO_2 in 120 min, while *Aspergillus niger* could not be inactivated with or without the catalyst after 240 min [Seven *et al.,* 2004].

On the other hand, Maneerat and Hayata (2006), using a UV-A (315-400 nm) black light (1 mW/cm^2) to irradiate suspensions of *Penicillium expansum* and TiO_2 in 72 h, found that the TiO_2 photocatalytic reaction reduced conidial germination of the fungus pathogen. They also demonstrated that the ability of photocatalytic treatment to suppress *P. expansum* growth was correlated to the amount of TiO_2 added. Lonnen *et al.* used immobilized TiO_2 on acetate sheets exposed to an artificial solar simulator with an irradiance exposure of 870 W/m^2 in the 300 nm–10 mm range (200W/m^2 in the 300 – 400 nm UV-A range) [Lonnen *et al.,* 2005].

This work corroborates our results, as in almost all experiments solar photocatalytic performance was better than solar-only disinfection. They also found more than a 5-log reduction of *C. albicans* and *F. solani* with 4 h of photocatalytic treatment. This means the UV-A dose used by these authors was 2880 kJ/m^2, which is 5 to 30 times higher than in our work. This difference is probably due to the fact that Lonnen and coworkers used a UV lamp with UV-A irradiance higher than the 28 W/m^2 average solar UV-A irradiance

recorded at the PSA (Almería, Spain) during our experiments. They also use a higher initial concentration of microorganisms (10^5 CFU/mL) than we did. Lonnen *et al.,* demonstrated that the solar disinfection of 10^5 CFU/mL of *C. albicans* required 6 hours exposure and *F. solani* 8 h with the same lamp. Theses exposures indicate than the global radiation doses required were 18972 and 25056 kJ/m^2 respectively, which are on the same order of magnitude, although somewhat higher, than we found under natural solar irradiation for experiments with a lower initial spore concentration (10^3 CFU/mL).

Interaction between TiO_2 and fungus spores: The photocatalytic experiments were also analyzed from the point of view of the catalyst–target organism interaction. For this the fungus suspensions were observed with an optical microscope. **Figure 1.8 a, b** shows *F. equiseti* macroconidia during the experiments. **Figure 1.8 a** shows a macroconidia before inactivation, and **Figure 1.8 b** after 5 h of photocatalytic treatment. **Figures 1.9 a** and **1.9 b** confirm a similar result for *F. oxysporum* spores. Before the process, the macroconidia are well defined spores, whereas after 5 h of TiO_2 photocatalysis, the TiO_2 aggregates can be seen attached to the surface of the spore and the cell wall is not as well defined. As the pH of the water was between 6 and 4 during the experiment, and the PZC of TiO_2 is around pH 7 [Fernández-Ibáñez *et al.,* 2000, 2003], the TiO_2 particles in the water are positively charged. The tendency of the titania aggregates to approach and attach themselves to the macroconidia cell wall may be explained by the opposite surface charges of the titania particles and the fungus spore surface. In fact, the fungus cell wall is expected to be negatively charged as is the case of many other microorganisms in water with a few exceptions [Van Der Mei *et al.,* 1998].

Nevertheless, the molecular composition of the *F. equiseti* macroconidia surface is currently unknown and additional research is necessary to identify the molecules that are responsible for the surface properties observed. The chemical composition of microorganism cell membranes has a strong impact on the physicochemical surface properties, and therefore, on interfacial phenomena, such as aggregation and adhesion [DUV-Al *et al.,* 2005].

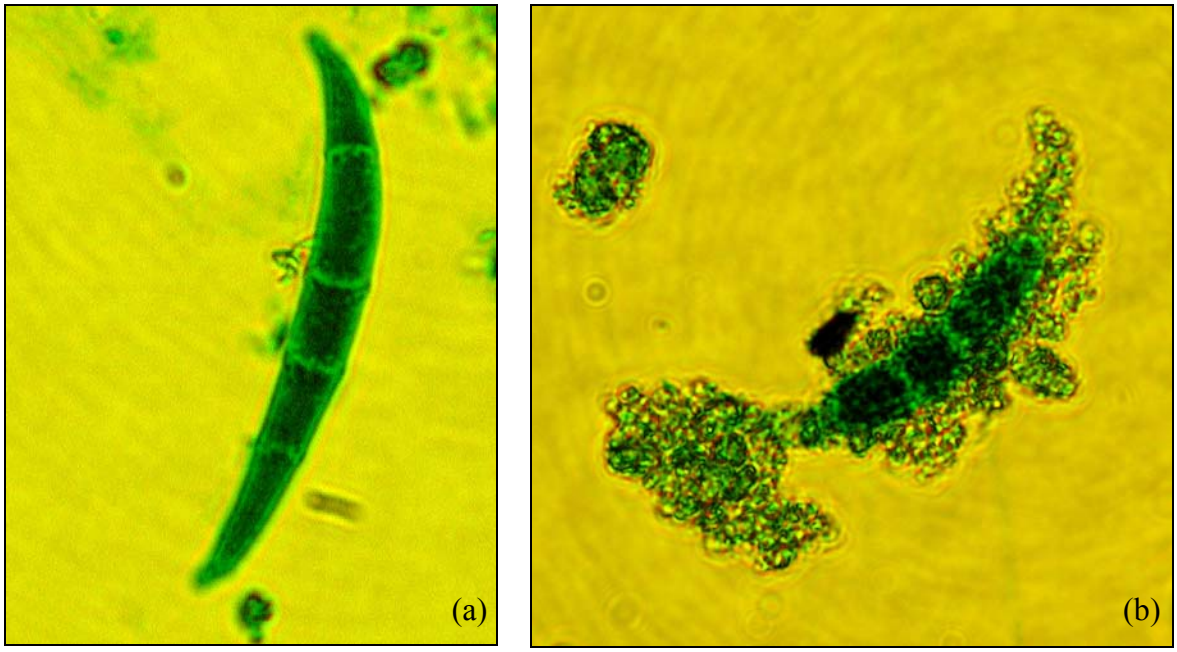

Figure 1.8 Macroconidia of *F. equiseti* stained with Malachite green before (a) and after 5 hours of solar photocatalytic treatment (b) (x1000).

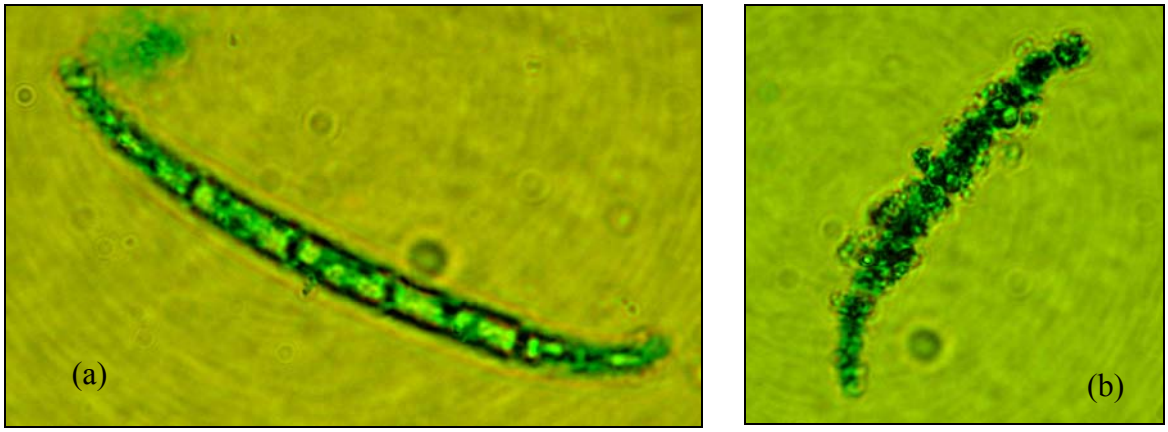

Figure 1.9 *F. oxysporum* macroconidia stained with Malachite green before (a) and after 5 hours of solar photocatalytic treatment (b) (x1000).

A similar tendency was observed by Nadtochenko *et al.,* They demonstrated the attachment of TiO_2 aggregates to the surface of *E. coli* cells; they attributed also this fact to the different surface charge of both, catalyst and cell wall [Nadtochenko *et al.,* 2004]. Our observations of fungal spores in presence of TiO_2 in the dark demonstrate the same behaviour (photos not shown).

There is an abundance of literature on the influence of different metals, radicals, and salts on the surface charge of microorganisms and, thereby, on their interaction with other matter [Van der Mei *et al.*, 1994; DUV-Al *et al.,* 2005; Gaboriaud *et al.,* 2006]. Like other colloidal particles, bacteria and other microorganisms have a surface charge that originates in the ionization of surface molecules and the adsorption of ions from the solution. Bacteria cell walls and membranes contain numerous proteins, lipid molecules, teichoic acids, and lipopolysaccharides which give them a characteristic charge [Szumski *et al.,* 2005].

The surface properties of *Aspergillus fumigatus* and *A. nidulans* conidia were studied by Girardin *et al.* These authors evaluated the hydrophobicity, Lewis acid-base (i.e. electron donor/acceptor) characteristics and electrostatic charge of various types of spores of both fungi. They found that the physico-chemical differences between *A. fumigatus* and *A. nidulans* conidia can be attributed to differences in the composition of the outer conidia cell-walls of the two species [Girardin *et al.,* 1999].

Dunlap *et al.,* report results on the physicochemical surface properties of blastospores of the entomopathogenic fungus *Paecilomyces fumosoroseus*. They made zeta potential measurements to quantify the surface charge and determine the zero potential of the blastospores. Their results showed that *P. fumosoroseus* blastospores have a basic monopolar and hydrophilic surface. Blastospores were negatively charged under neutral conditions at an isoelectric point of 3.4 [Dunlap *et al.,* 2005]. Some studies of spore aggregation have been published, such as the one by Dynesen and Nielsen, who studied the influence of fungus conidiospore wall hydrophobicity on its agglomeration and pellet formation, and electrophoretic mobility in a wild strain of *A. nidulans*.

They concluded that the electrical charge and hydrophobicity of the conidiospores affects pellet formation, but that their agglomeration cannot be ascribed to these factors alone [Dynesen and Nielsen, 2003]. Klotz studied the contribution of electrostatic interaction to the adherence of *C. albicans* and other *Candida* sp. by mixing cationic or anionic exchange resins possessing free $-COO^-$ or $-NH^{+4}$ groups, respectively, on their surface. It was found that most of the *Candida* studied had electrostatic surface charges [Klotz, 1994]. As good adsorption of the catalyst provides good conditions for the photocatalytic disinfection

process in heterogeneous photocatalysis [Herrmann, 2005], these photos (**Figure 1.8** and **1.9**) are promising for photocatalytic disinfection.

Conclusions

- Solar-only disinfection and solar photocatalytic disinfection processes are effective for the inactivation of spores of the soilborne fungal phytopathogens *F. equiseti, F. anthophilum, F. verticillioides, F. oxysporum* and *F. solani*. Except for *F. equiseti*, the fungi studied were readily inactivated under natural solar irradiation with and without TiO_2 after 6 hours of exposure. In all cases, under similar experimental conditions, solar photocatalytic inactivation is much faster than for solar-only disinfection.

- *F. equiseti* was found to be the most resistant *Fusarium* species evaluated in this work. While the rest of the *Fusarium* species studied were inactivated by both methods (solar-only disinfection and solar photocatalysis), *F. equiseti* resisted for 6 hours of natural solar irradiation (UV-A: 10.1 kJ/L, 612.1 J/m^2). *F. equiseti* also showed the lowest inactivation rate in the presence of TiO_2.

- The order of resistance of the five fungi studied was: *F. equiseti* >*F. anthophilum* > *F. solani* > *F. oxysporum* > *F. verticillioides* with solar photocatalysis, and *F. equiseti* > *F. anthophilum* > *F. verticillioides* > *F. solani* > *F. oxysporum* with solar-only disinfection.

- TiO_2 showed a strong tendency to attach to the fungus spore surface, which is very positive for the effectiveness of the photocatalytic reaction and therefore for its application in solar disinfection systems.

Acknowledgements

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IV.2. Response of *Fusarium equiseti* spores to solar photocatalysis

Abstract

Common irrigation water disinfection methods are not very effective in controlling the appearance of phytopathogens. These methods may be unable to inactivate all types of pathogens or even become phytotoxic themselves. Solar disinfection by photocatalysis is a promising irrigation water treatment method for destroying phytopathogens without the drawbacks of conventional disinfection methods. This paper reports on the ability of solar photocatalysis to inactivate *F. equiseti* spores. Experiments in water measured the viability of two types of wild *F. equiseti* spores (macroconidia and chlamydospores) in 200-mL-flasks after exposure to solar radiation in the presence of titanium dioxide powder (TiO_2) as a photocatalyst. *Fusarium* spore suspensions were exposed to natural sunlight for 5-6 hours of full radiation in Southern Spain. Spore viability was evaluated by culturability measurements using acidified malt agar. Solar photocatalytic results showed that *F. equiseti* spores were sensitive to the treatment. Five hours of solar exposure reduced spore viability from 600 (±8) CFU/mL to 3 (±2) CFU/mL. Experiments with selective suspensions of chlamydospores and macroconidia separately demonstrated that chlamydospores are more resistant than macroconidia, since 5 h exposure reduced macroconidia suspensions from 2950 (±40) CFU/mL to 2(±2) CFU/mL and chlamydospores diminished from 2200 (±30) CFU/mL to 50 (±10) CFU/mL. These results confirm that solar photocatalytic disinfection of contaminated water can be effective against fungal contamination of water sources.

Introduction

In the last decade, intensive hydroponic agriculture has increased in certain regions of the world. These systems are also growing in semiarid areas with high solar radiation and enough underground water. The advantage of using such systems is mainly savings in water and fertilizer. Various organic and inorganic inert substrates, like perlite [Vázquez

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Authors: Sichel, C., de Cara, M., Tello, J., Fernández-Ibáñez, P.

and Carpena-Ruiz, 2005], rockwool [Le Bot and Adamowicz, 2006] or coconut fiber [Raviv, 2005], can be used in them. The liquid nutrient solutions used in soilless culture systems create a very different environment, and consequent pathosystem, in the plant root zone from traditional agriculture. Therefore, this system has given rise to a new issue related to water-borne diseases [Runia, 1994] which is difficult to address. Several chemical fungicides, such as etridiazole, have been demonstrated to become phytotoxic, and chlorine, the most widely used disinfectant, is not only phytotoxic also, but is not even always effective.

Phytopathogen control in such horticultural systems involves monitoring the main species responsible for contamination of the nutrient solution and disinfection. New methods focusing on this issue include UV-C (254 nm) disinfection. Runia used UV-C to treat water with *Phytophthora* and *Phytium* [Runia and Boonstra, 2001]. Nevertheless this treatment is strongly limited by light penetration in the solution, and therefore, its efficacy is also limited. Other pioneering publications have recently demonstrated that TiO_2 photocatalysis, an Advanced Oxidation Process (AOP), can be used as an alternative treatment for pathogens and phytopathogens present in the water [Sichel *et al.,* 2007 a, Maneerat and Hayata, 2006].

AOPs are based on physicochemical processes that produce powerful transitory species in situ, mainly hydroxyl radicals ($^{\bullet}OH$), by using appropriate energy, and are very effective for oxidizing organic matter. Under specific conditions, hazardous compounds are removed and converted to CO_2, H_2O and innocuous mineral salts [Augugliaro *et al.,* 2006]. Heterogeneous photocatalysis is an AOP using a wide-band-gap semiconductor, usually titanium dioxide (TiO_2), which has been proven very efficient for the decontamination of water containing hazardous pollutants [Fujishima *et al.,* 2000].

The series of surface chemical reactions occurring during this process are well known (**Eqs. 2.1-2.7**). When the semiconductor particle is photoexcited by a source of photons with enough energy, i.e., higher than the semiconductor band-gap (E_G, $\lambda < 385$ nm for

TiO_2), an electron from the valence band is promoted to the conduction band, leaving an electron vacancy or hole in the valence band, thus generating electron/hole pairs. Both reductive and oxidative processes may occur at/or near the surface of the semiconductor particle. When the system is in the presence of oxygen, the oxygen molecules are able to scavenge conduction band electrons forming superoxide ions ($O_2^{\bullet -}$) and hydroperoxyl radicals ($HO_2^{\bullet}$), This avoids electron/hole recombination (**Eq. 2.2**) and prolongs the lifetime of photogenerated holes, permitting the generation of hydroxyl radicals in the presence of adsorbed water molecules (**Eq. 2.4 and 2.7**). In aqueous solutions, oxidation of water to hydroxyl radicals seems to be the predominant pathway, although the role of other oxidizing species remains unclear [Augugliaro *et al.,* 2006]. The key characteristic of this process is the non-selective property of hydroxyl radicals, which attack any organic matter present in the medium until their complete mineralization.

$$TiO_2 + h\nu\ (>E_G) \rightarrow TiO_2\ (e^- + h^+) \quad \textbf{(Eq. 2.1)}$$

$$TiO_2\ (e^- + h^+) \rightarrow TiO_2 + (E_{th} + h\nu') \quad \textbf{(Eq. 2.2)}$$

$$TiO_2\ (h^+) + RedX_{ads} \rightarrow TiO_2 + RedX_{ads}^{\bullet +} \quad \textbf{(Eq. 2.3)}$$

$$TiO_2\ (h^+) + H_2O_{ads} \rightarrow TiO_2 + {}^{\bullet}OH_{ads} + H^+ \quad \textbf{(Eq. 2.4)}$$

$$TiO_2\ (h^+) + {}^{-}OH_{ads} \rightarrow TiO_2 + {}^{\bullet}OH_{ads} \quad \textbf{(Eq. 2.5)}$$

$$TiO_2\ (e^-) + O_2 \rightarrow TiO_2 + O_2^{\bullet -} \quad \textbf{(Eq. 2.6)}$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet} \quad \textbf{(Eq. 2.7)}$$

The first work on photocatalytic disinfection was done by Matsunaga *et al.,* [Matsunaga *et al.,* 1985], who demonstrated that titanium dioxide irradiated by a UV-A lamp is able to inactivate a suspension of *E. coli* in water. Since then, a number of related contributions have reported the disinfection capacity of the titanium dioxide semiconductor used as the photocatalyst [Blake *et al.,* 1999; Malato *et al.,* 2007; McCullagh *et al.,* 2007]. Most of the work on photocatalytic disinfection has focused on the mechanisms and analysis of the main parameters using *E. coli* bacteria as the model microorganism [Herrera Melián *et al.,* 2000; Rincón and Pulgarin, 2004].

In the work referred to above [Matsunaga *et al.,* 1985], the photooxidation of Coenzyme A (CoA) in *Lactobacillus acidophilus* (bacteria), *S. cerevisiae* (yeast) and *E. coli* (bacteria)

was demonstrated in suspensions of irradiated TiO_2. Decreased CoA in those cells caused their metabolic activity to diminish leading to cell death. Other recent publications have studied the sites on the cell damaged by oxidation and their contribution to cell death in *E. coli*, contributing to a better understanding of the bactericidal activity of photo-excited titanium dioxide [Huang *et al*., 2000; Sunada *et al.,* 2003; Kiwi and Nadtochenko, 2005]. They found primary oxidative damage to the cell wall (because it is the first microorganism surface coming in contact with the photocatalyst) due to peroxidation of the polyunsaturated phospholipid component of the lipid cell membrane, leading to a loss of essential cell functions, e.g., respiratory activity, and later to cell death.

There is currently considerable interest in the application of TiO_2 photocatalysis to water disinfection. However, there have been few contributions to photocatalytic disinfection of fungi [Wolfrum *et al.,* 2002; Seven *et al.,* 2004; Lonnen *et al.,* 2005; Maneerat and Hayata, 2006]. Maneerat and Hayata showed the antifungal activity of irradiated TiO_2 (powder and coated plastic film) on *Penicillium expansum* in fruit root and discussed the potential of this treatment for post-harvest disease control. Lonnen *et al.,* demonstrated photocatalytic inactivation of *C. albicans* and *F. solani* in batch-mode using simulated solar radiation. Seven *et al.,* studied the photocatalytic disinfection of several microorganisms, among them *A. niger* and *C. albicans*.

We had previously studied photocatalytic treatment of water contaminated with five wild strains of the *Fusarium* genus (*F. equiseti, F. oxysporum, F. anthophilum, F. verticillioides,* and *F. solani*) using natural sunlight as the source of UV-A photons [Sichel *et al.,* 2007 a]. Exposure times from 1 h to 6 h were enough to inactivate a fungus concentration of 10^3 CFU/mL in water using powdered Degussa P25 TiO_2 (Degussa, Germany) suspended in water in 200-mL batch photoreactors. We also found certain sensitivity of these spores to solar radiation alone, although the photocatalytic disinfection times were shorter and disinfection better than for solar-only disinfection. The mechanisms governing solar disinfection are different from photocatalysis. During exposure to sunlight, the UV photons (UV-A and UV-B, around 4% of the solar spectrum in Spain) penetrate the samples and combined with the thermal effect, induce a loss of viability in the microorganism or spore suspensions (McGuigan *et al.,* 1998). Solar disinfection was

demonstrated to be effective for a wide range of microorganisms under lab conditions [Kehoe *et al.,* 2004; Lonnen *et al*., 2005].

Our previous work showed varying resistances of different types of spores to the solar photocatalytic treatment, with *F. verticillioides* (microconidia producer) the most susceptible to TiO_2 photocatalysis and *F. equiseti* (macroconidia, and chlamydospores) the most resistant. The results of such work suggested that structural differences in spore may determine resistance [Sichel *et al.,* 2007 a]. This work focused on solar photocatalytic water disinfection of different types of spores of *F. equiseti* strains. *F. equiseti* had recently been isolated from water from a river in Almería (southeast of Spain), and from deep soil in the Mediterranean Sea [Palmero *et al.,* 2008], and its pathogenicity has been proven for different species [Palmero *et al.,* 2008; Punja *et al.,* 2007]. As it is known that this species produces both macroconidia and chlamydospores, particular emphasis is placed separately on experimental response of *F. equiseti* chlamydospores and macroconidia to the photocatalytic treatment.

Materials and Methods

F. equiseti spore preparation & sampling: The fungal strains used were original wild isolates recovered from the Andarax River (Almería, Spain) in the collection of the University of Almería Plant Production Department. Their pathogenicity has previously been proven to cause "damping-off" on tomato, melon, *Brassica* sp. and barley [Palmero *et al.,* 2008]. Originally, about 200 isolates of *F. equiseti* were taken and transferred to the CLA medium (Difco, USA). After 1 month of incubation under 24-hour-a-day UV-C radiation, three isolates were selected. One only produced macroconidia, and another produced chlamydospores exclusively in terminal or intercalary hyphal cells. These two strains preserved the ability to produce only one type of spore after being transferred three times to CLA. The third strain was a common *F. equiseti* strain that produced mainly macroconidia but also chlamydospores.

The strains were transferred to the sporulation medium, agar containing sodium chloride in Petri dishes [Nelson *et al.,* 1983] exposed to UV-C radiation from a mercury lamp (40 W) for 15-30 days at 25 ºC to produce the required number of spores (ca. 10^5 CFU/dish).

Spores and hyphal fragments were detached from the agar by washing the plates with distilled, autoclaved water (121 ºC, 60 min). Chlamydospores from the chlamydospore-only strain were separated by titrating the whole mycelium and agar contained in the Petri dishes, with distilled sterile water, and later recovering the supernatant after 10 min. sedimentation. Suspensions were placed in bottle-reactors to obtain initial spore concentrations between 10^2-10^4 CFU/mL for different experiments. Concentrations were determined by direct counting using a Neubauer plate (Brand, Germany).

Solar experiments: All experiments were performed in 250-mL DURAN-glass (Schott, Germany) batch bottle-reactors, in which the initial *F. equiseti* strain spore dilutions were mixed. Two types of disinfection were tested. a) Solar disinfection, by exposing bottles containing *F. equiseti* to sunlight, and b) Solar photocatalytic disinfection, by previously adding TiO_2 P25 (Degussa, Germany) to the fungal inoculation. TiO_2 was used as received and stirred into the water as a colloidal suspension. Afterwards, the fungal suspension was inoculated and bottles were exposed to sunlight. The optimal catalyst concentration was 35 mg/L, as previously reported [Sichel *et al.,* 2007 a]. This concentration permits the use of 99% of incoming solar UV energy, which is the spectral range that excites the catalyst.

After mixing *F. equiseti*, bottles were covered with glass covers (Schott, Germany) to allow the solar radiation to enter the bottle-reactor from all directions. The bottles were stirred with magnetic stirrers at 100 rpm during the experiment. All experiments were performed in triplicate (three bottles per treatment), under natural sunlight at the Plataforma Solar of Almería, Spain (latitude: 37.0909 ºN, longitude: 2.357 °W). The solar photocatalytic and solar disinfection tests were carried out simultaneously and the control bottles (with and without TiO_2) were stored under the same conditions, but in the dark. The results of all three replicates were highly reproducible. The experimental error was around 10% when the concentration of fungus was over 50 CFU/mL, and between 50 and 2 CFU/mL, the error obviously increased, as expected for the plate count technique.
Thermal inactivation of fungi was discarded since temperatures during all experiments were always below 35 °C. Nevertheless, the control samples in the dark were kept at the same temperature as the solar experiments to avoid different thermal effects in illuminated

and dark samples. All solar experiments were conducted under similar irradiation conditions for exposure times of 5 to 6 hours.

After exposure, three 50-μL samples were recovered from each bottle and plated out using the spread-plate technique on acidified malt-extract agar (Sigma Aldrich, USA). After two days of incubation at 28 ºC in the dark, the number of colonies was counted. When fewer than 10 CFU per dish were observed, 500 μL of the sample were plated with a 2 CFU/mL detection limit. The statistical analysis gave the average colony concentration and the error at a 95% confidence level for all results reported. Conductivity remained between 650 and 710 $\mu S \cdot cm^{-1}$, which is high compared to distilled water. This is due to ions entering the water during spore preparation. The initial pH was always around 6 in bottles.

Using this methodology, three types of experiments could be performed: 1) Comparison of solar and solar photocatalytic disinfection to inactivate a common *F. equiseti* strain, for a 10^3 CFU/mL spore suspension. 2) Inactivation of three different dilutions of *F. equiseti* chlamydospores using photocatalytic disinfection. 3) Comparison between photocatalytic disinfection of an *F. equiseti* macroconidia suspension vs. a chlamydospore suspension.

Optical Phase-Contrast and Clearfield Microscopy: A Model Eclipse 50i Nikon (Japan) optical microscope was used for counting microorganisms stained with 50 mg/mL malachite green and TiO_2 adsorption monitoring. Pictures were taken with a Nikon Coolpix 8400 camera, in clear field mode for adsorption and in phase contrast mode for Neubauer counting.

Results

Inactivation of Fusarium equiseti with solar photocatalysis and solar disinfection: Solar photocatalytic treatment with TiO_2 induces gradual inactivation of *F. equiseti* spores (**Figure 2.1**). Nevertheless, direct action of sunlight shows a slight fungicidal effect as shown in **Figure 2.1**, although this is much less effective than photocatalysis.

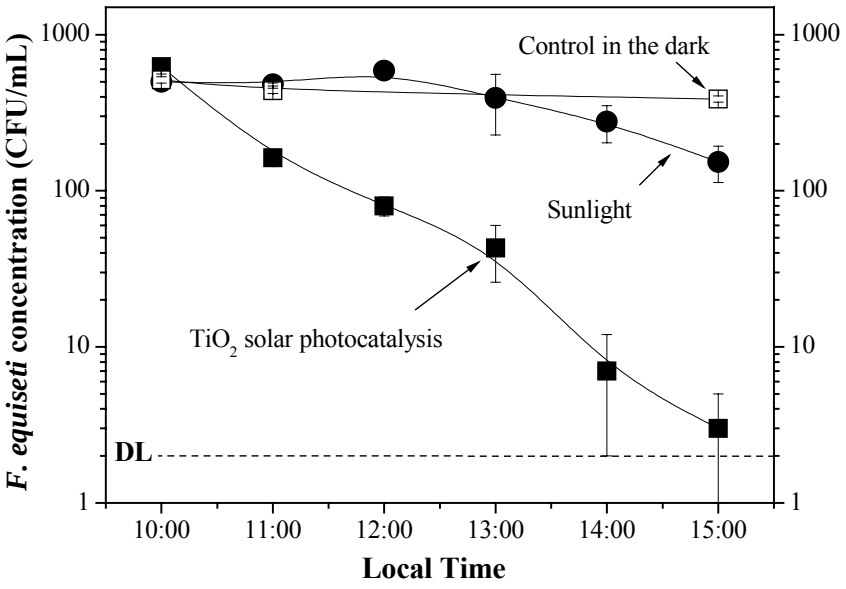

Figure 2.1 Effect of TiO_2 (35 mg/L) photocatalysis versus solar-only on *F. equiseti* viability. Initial concentration of spores 600 CFU/mL. Evolution of spore concentration with (–■–) and without TiO_2 (–●–), and dark control samples (–□–). December 2006.

While the TiO_2 photocatalytic treatment causes a 3-log decrease, the solar-only decrease is not quite 0.3 log. The fungal suspension in contact with the TiO_2 slurry shows complete disinfection within 5 h of solar irradiation. It should be mentioned that this series of experiments was done with spore suspensions having mostly macroconidia.

Influence of the fungal spores on the survival of F. equiseti: To detect the difference in behaviour of chlamydospores at different concentrations, three initial concentrations of spores were selected for these experiments (10^4, 10^3, and 10^2 CFU/mL). **Figure 2.2** shows the evolution of spore viability during photocatalytic treatment. All the concentrations decreased around 1-log during the first three hours of solar exposure, and then, half-way through exposure, became almost constant at spore concentrations of around 600, 100 and 10 CFU/mL for the three cases evaluated, respectively.

The disinfection curves in **Figure 2.2** were quite different from **Figure 2.1** and also from the typical decrease in photocatalytic disinfection found for other *Fusarium* spores in our previous work [Sichel *et al.,* 2007 a, b]. This unusual behaviour was observed on

completely sunny days in February 2007, when the experiments were carried out. Since lack of solar radiation can be excluded as the reason for recovery of the *Fusarium* population, this disinfection activity was attributed to the presence of mainly chlamydospores in the suspension, compared to mostly macroconidia and a few chlamydospores as shown in **Figure 2.1**.

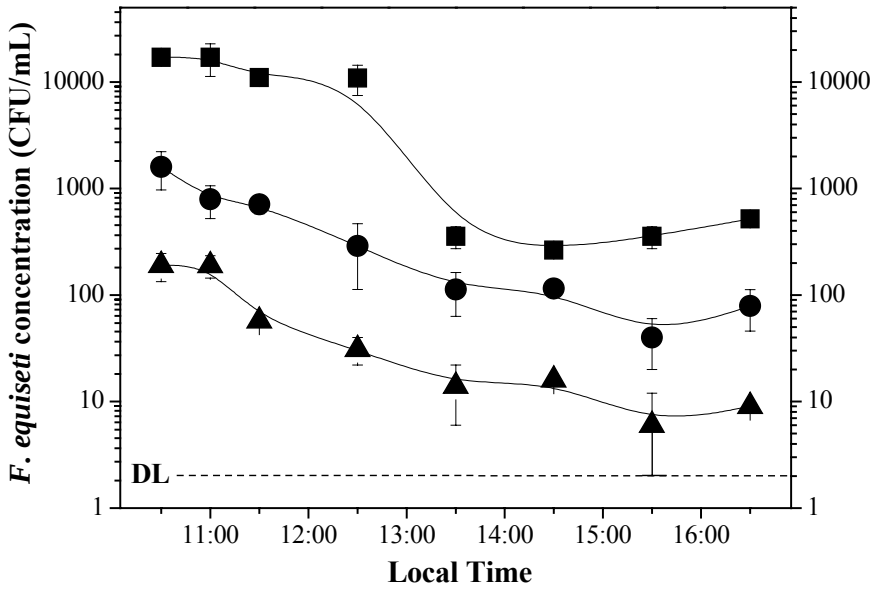

Figure 2.2 TiO_2 photocatalytic treatment of *F. equiseti* chlamydospores. Evolution of spore viability at three initial spore concentrations: 18 000 (–■–), 1 700 (–●–), and 200 CFU/mL (–▲–). February 2007.

This led us to believe that chlamydospores might be more resistant to treatment than macroconidia. To confirm this, another series of experiments were carried out with different types of *F. equiseti* producing macroconidia and chlamydospores separately. The solar photocatalytic tests were done under the same conditions.

Figure 2.3 shows the resulting viability of different spores. As observed, the fungal suspensions containing macroconidia were disinfected better than those with chlamydospores. Five hours of exposure to natural solar radiation in the presence of TiO_2 caused the macroconidia concentration to decrease from 2950 (±40) CFU/mL to 2 (±2) CFU/mL and chlamydospores diminished from 2200 (±30) CFU/mL to 50 (±10) CFU/mL.

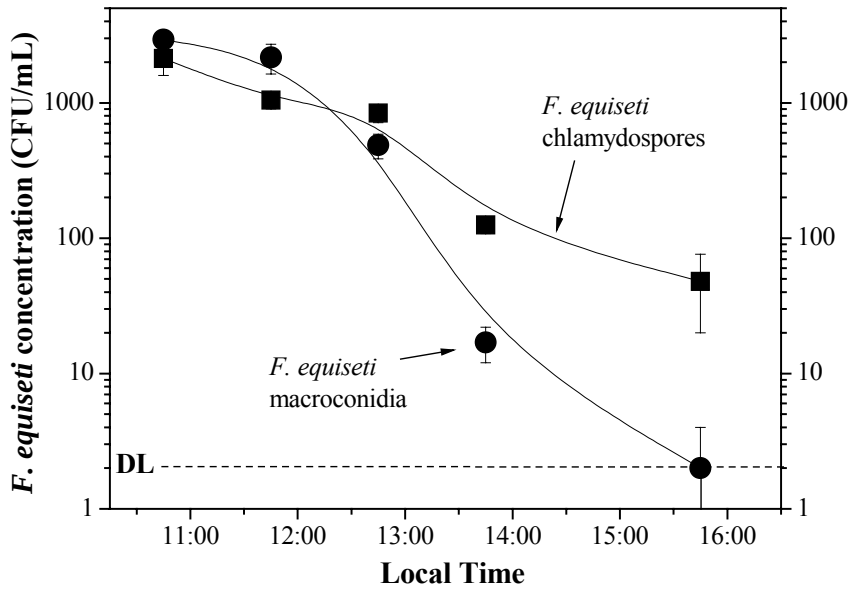

Figure 2.3 TiO_2 photocatalytic inactivation of *F. equiseti* chlamydospores (–■–), and *F. equiseti* macroconidia (–▲–). February 2007.

Recovery of spores after photocatalytic treatment: Before and after the photocatalytic treatment, the suspensions of *F. equiseti* were studied with an optical microscope for interaction between spores and catalyst. The cause of such interaction, among others, is assumed be electrical, since both system components (spore and catalyst) have an effective surface electrical charge [Sichel *et al*., 2007 a].

Figures 2.4 a and **2.4 b** clearly show TiO_2 aggregates attached to the surface walls of *F. equiseti* spores. These images also show how the internal spore membranes and external walls break up under the strong effect of photocatalytic treatment. In contrast, **Figures 2.4 c** and **2.4 d** show a completely different result for *F. equiseti* chlamydospores. These spores were able to resist the treatment and were still viable one day later. The cell wall is still well defined and the spores germinated. However, some chlamydospores were observed to be attacked by the catalyst, as seen in **Figure 2.4 e**. This observation confirms that, as **Figure 2.3** suggests, although chlamydospores are more resistant to the treatment, they can still be inactivated by solar photocatalysis.

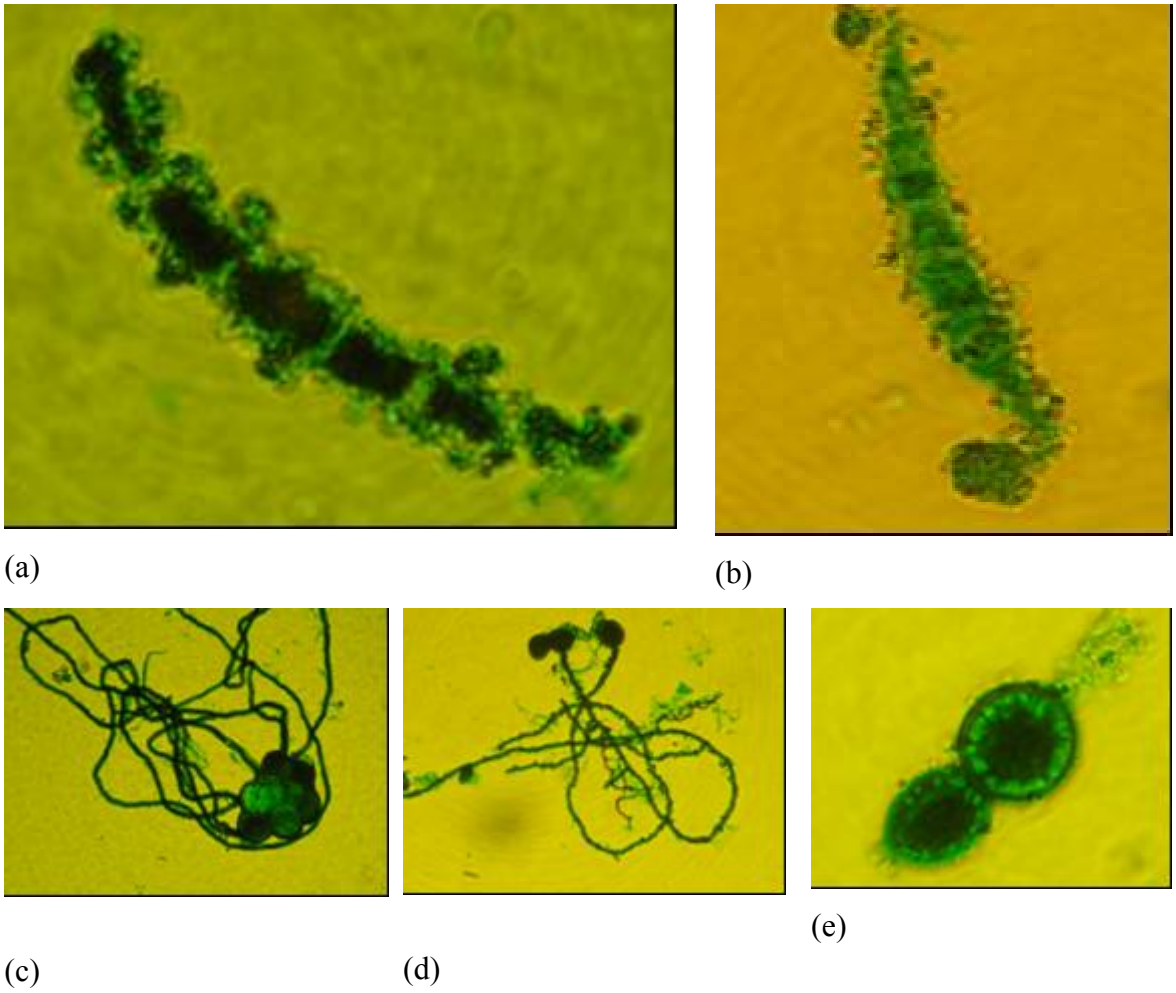

Figure 2.4 *F. equiseti* macroconidia (a, b) and chlamydospores (c, d, e) stained with Malachite green one day after the photocatalytic treatment (x1000).

Discussion

Our previous work Sichel *et al.,* 2007 demonstrated similar photocatalytic inactivation of other *Fusarium* species. In this work, photocatalytic treatment with TiO_2 was found effective for the disinfection of water containing *F. equiseti, F. oxysporum F. anthophilum, F. verticillioides*, and *F. solani*. Complete photocatalytic disinfection of all *Fusarium* species was observed within 1-5 h of solar exposure.

Specific publications on the photocatalytic inactivation of bacteria indicate that oxidative damage first occurs on the external cell wall, where the surface of the catalyst (TiO_2) comes into contact with whole cells (Kiwi and Nadtochenko 2004). The reactive oxygen

species directly involved in photokilling, which may be not only $^{\bullet}OH$, but also $O_2^{\bullet-}$, and H_2O_2, remains uncertain [Sunada *et al.,* 2003]. Nevertheless, Cho *et al.,* confirmed the key role of $^{\bullet}OH$ in *E. coli* inactivation during photocatalysis [Cho *et al.,* 2004]. The spores used for this work are mainly differentiated by their cell wall. In *F. equiseti* microconidia spores are absent [Gerlach and Nirenberg 1982; Leslie and Summerell, 2006), and macroconidia are described as strongly septate, and thick-walled [Nelson *et al.,* 1983]. Chlamydospores are common in many strains of *F. equiseti* [Nelson *et al.,* 1983] and this spore is defined as a viable, asexually produced accessory spore resulting from the structural modification of a vegetative hyphal segment(s) or conidial cell possessing a thick wall mainly consisting of newly synthesized cell wall material [Schippers and van Eck, 1981]. Thickness of the chlamydospore wall seems to be an important factor for survival in natural soils in the absence of host plants [Warcup, 1955].

Electron microscope studies of *F. solani* f. sp. *cucurbitae* chlamydospores formed in natural soil suggested that the newly formed chlamydospore cell wall substance is more resistant to lysis than the walls of macroconidia [Old and Schippers, 1973]. The chemical composition of macroconidium and chlamydospore walls is similar and consists mainly of glucosamine, glucose, mannose, galactose, amino acids and glucuronic acids, in different proportions [Schippers van Eck, 1981]. It is also known that during chlamydospore formation, electron-dense material is deposited in the macroconidial cell wall. This material has a melanin-like nature, and melanin is known to enhance resistance of fungal cell walls to microbial lysis [Kuo and Alexander, 1967].

Perhaps this melanin-like material also enhances the resistance to light penetration to inner layers of chlamydospore walls. Other studies, on *F. oxysporum*, show that chlamydospores have a thick microfibrilar wall inside the hyphal wall during chlamydospore formation, which consists of randomly oriented microfibrils. X-ray diffraction analyses show that these are chitin microfibrils thought to be embedded in a carbohydrate and protein matrix which constitutes the bulk of the wall [Schippers van Eck, 1981].

F. equiseti has previously been demonstrated as the *Fusarium* species most resistant to solar photocatalysis [Sichel *et al.,* 2007 a], probably due to the formation of more robust

spores than those formed by the other fungi. *F. solani* and *F. oxysporum* produce macroconidia but very few chlamydospores, while the used *F. verticillioides* and *F. anthophilum* strains generate microconidia and very few macroconidia [Nelson *et al.,* 1994].

During the experiments, the pH dropped from 6 to 4, while pH remained constant in the solar controls without TiO_2. This drop can be attributed to the compounds generated during photocatalysis, since it is not due to TiO_2 reaction with water [Sichel *et al.,* 2007 a]. Like other colloids, microorganisms usually have a surface charge, especially the cytoplasmatic bacterial membrane, which is formed by proteins, lipids, acids, and lipopolysaccharides, which give them a characteristic negative charge [Szumski *et al.,* 2005].

Several studies have reported on the surface properties of the spores of different fungus species, such as the negative surface charge of *Paecilomyces fumosoroseus* [Dunlap *et al.,* 2005], hydrophobicity of *A. nidulans* conidiospores [Dynesen and Nielsen, 2003], and the electrostatic interaction of *C. albicans* and other *Candida* sp. [Klotz, 1994]. On the other hand, the titanium dioxide particles in water also have a surface charge which depends on the pH of the medium. TiO_2 particles and agglomerates have a positive charge at pH 6 [Fernández-Ibáñez *et al.,* 2003], which was the pH during the experiments. Given the opposite charges of spores and catalyst, a strong hetero-aggregation effect (between different kinds of colloids) is expected during the experiments (**Figures 2.4 a**, and **2.4 b**).

These are the best photocatalytic treatment conditions, since adsorption of the substance to be degraded on the catalyst surface is a pre-requisite for the photocatalytic reaction [Herrmann 2005]. This explains the good disinfection rate during photocatalytic inactivation of *F. equiseti* macroconidia and the images observed after treatment. Nevertheless, disinfection of chlamydospores is not so good. This was attributed above to the robustness of the chlamydospore structure, and **Figures 2.4 c** and **2.4 d** also show how they recovered from the treatment.

We would like to emphasize that our results open a promising new way to disinfect water containing *F. equiseti*, which has recently been reported as a pathogen in ginseng, inducing

reddish-brown sunken lesions on roots [Punja *et al.,* 2007], and seed decay and brown lesions on roots and hypocotyls of various leguminosae [Punja *et al.,* 2007]. Several strains of *F. equiseti* isolated from water in the Andarax River are pathogenic in melon, tomato, barley and *Brassica* sp. [Palmero *et al.,* 2008].

Conclusions

- Solar photocatalytic disinfection is effective for the inactivation of *F. equiseti* microconidia and macroconidia, which were inactivated after 5 hours of photocatalytic treatment under natural solar radiation. This is a promising result for future applications of solar energy in new disinfection systems.

- *F. equiseti* chlamydospores were found to be more resistant than macroconidia to the photocatalytic reaction, since concentrations of chlamydospores were not completely abated until zero or detection limit. Microscope observation also confirmed chlamydospore recovery and germination after treatment.

Acknowledgements

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IV.3. Effects of experimental conditions on *E. coli* survival during solar photocatalytic water disinfection

Abstract

Results of photocatalytic disinfection of *E. coli* K12 in water in a compound parabolic collector (CPC) solar reactor are reported. The aim of the study is to quantify the influence of operating parameters, such as flow rate, water quality and bacterial concentration, on bacterial viability in solar photocatalysis and in the dark. The catalyst used was an industrial titanium-dioxide-coated paper matrix fixed on a tubular support in the focus of the CPC. Addition of TiO_2 notably improved solar-only disinfection up to 6 logs disinfection in 90 min. Between 10 and 2 L/min, photocatalytic disinfection effectiveness tended to increase with decreasing flow rates. In dark experiments, inactivation of 99% of viable *E. coli* cells in distilled water was detected after 90 minutes of recirculation at 10 L/min in the CPC reactor. A detailed study of bacterial viability in the solar reactor in the dark was therefore performed, varying flow rates, initial concentrations and osmolarity. It was found that bacterial viability in the reactor strongly depends on all the parameters examined, so that disinfection and dark inactivation overlap when working under low-osmolarity conditions and low bacterial concentrations.

Keywords: osmotic stress, mechanical stress, solar reactor, photocatalytic disinfection, *E. coli*.

Introduction

A growing number of countries around the world have irrigation and drinking water supply problems. Many water sources are not only polluted by hazardous chemicals but also by pathogenic microorganisms and therefore, have to be disinfected before use. The most commonly used techniques for water disinfection are chlorination, heating and ozonation. The negative effect of chlorination is the appearance of trihalomethanes (THMs) as by products of its reaction with organic matter. It also gives drinking water an unpleasant taste

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Authors: Sichel, C., Blanco, J., Malato, S., Fernández-Ibáñez, P.

[Metcalf and Eddy, 2005; Bitton, 2005]. When used for irrigation, chlorine is often phytotoxic [Jarvis, 1998]. Other methods, e.g., ozonation, are either moderately expensive or involve high consumption of energy that is usually not sustainably produced [Metcalf and Eddy, 2005].

Often countries with the most serious safe water supply problems are among the sunniest in the world. This is why solar water disinfection methods for mainly rural areas, such as special solar drinking-water disinfection process called SODIS, have gained support in recent years. SODIS can be used as a sustainable small-scale, low-cost water treatment in transparent plastic bottles, and has been proven successful for the disinfection of a wide range of microorganisms [Joyce *et al.,* 1996; McGuigan *et al.,* 2006].

SODIS bases on the pasteurizing effect of solar radiation at temperatures higher than 40-45 ºC and on the synergistic interaction between the elevated temperatures and solar irradiation [McGuigan *et al.,* 1998; Berney *et al*., 2006 a]. Moreover, in some water matrixes sunlight can produce highly reactive oxygen species which attack bacterial cells and contribute to their inactivation [Gelover *at el.*, 2006; Rincón and Pulgarin, 2004 b]. Nevertheless, disinfection efficacy of the SODIS treatment can easily be affected by water turbidity, low irradiation intensity and regrowth of bacteria after the solar treatment, probably due to photo-repair mechanisms [Kehoe *et al.,* 2001, Rincón and Pulgarin 2004 b; Gelover *et al*., 2006].

On the other hand, heterogeneous photocatalysis with TiO_2, one of the new "Advanced Oxidation Technologies" (AOT), is a "clean", low-cost water treatment technology which can offer additional advantages in a wide range of applications [Herrmann, 2005]. These technologies are based on the production of $^{\bullet}OH$ radicals, and as they do not require the addition of chemical consumables, do not produce hazardous waste products. When the catalytic semiconductor TiO_2 is photoexcited with UV light at a wavelength equal to or lower than 390 nm, electron hole pairs are generated. In the presence of water and dissolved oxygen, hydroxyl radicals, which are very reactive and have a short lifetime, can be generated [Hoffmann *et al.*, 1995]. Photocatalytic disinfection has been demonstrated to be mainly controlled by temperature, catalyst physicochemical properties and

concentration, microorganism type and concentration, light intensity, exposure time and whether radiation is natural or simulated [Herrmann, 2005].

Application of TiO_2 to water treatment has been reviewed by Fujishima [Fujishima *et al.*, 2000] and more recently by Herrmann [Herrmann, 2005]. In recent years, scientific and engineering interest in TiO_2, especially photocatalysis for disinfection, has grown exponentially [Rincón and Pulgarin, 2003-2005; Malato *et al.*, 2007]. Its wide field of application has been reviewed by Blake [Blake *et al.,* 1999]. Some authors suggest that the cell membrane is the primary site of attack by the reactive hydroxyl radicals [Maness *et al*. 1999; Sunada *et al.,*2003]. Maness reported results that can be explained by peroxidation of the polyunsaturated phospholipid component of the lipid cell membrane leading to a loss of essential cell functions, e.g., respiratory activity, and in the end, to cell death. Most work has been done with TiO_2 powder in a slurry, some of it under natural sunlight, because reaction yields are better [Zhang *et al*., 1994; Block *et al*. 1997]. Disinfection with supported TiO_2 has reduced the need of post-treatment not only in the laboratory, but also in large solar reactors [Gelover *et al*., 2006; Rincón and Pulgarin, 2004 a; Fernández *et. al*., 2005].

Different types of solar photocatalytic and solar-only disinfection reactor configurations have been tested with promising results [Vidal *et al*., 1999 and Vidal and Díaz 2000; McLoughlin *et al.*, 2004]. CPCs have been found to be very effective for treatment of water polluted by chemicals as well as for disinfection [Malato *et. al.* 2002; Malato *et al*., 2004; Fernández *et al.,* 2005].

Photocatalytic disinfection in solar reactors is the last step in research before application to final disinfection systems, therefore optimisation of the disinfection yield and suitability of the treated water for its final use are major concerns at this stage of development. In many applications, rigorous study of photocatalytic disinfection requires the treatment water to have controlled chemical properties. Thus, the majority of work in this field uses artificially contaminated samples that are prepared with a standard microorganism in distilled water. With this medium, a significant negative affect on bacterial viability is not expected during various days [Kerr *et al*., 1999]. Very little work has been done with real

water sources, such as rivers, lakes and wells as reported by Rincón and Wist [Rincón and Pulgarin, 2004 b; Wist *et al.,* 2002].

This work starts out with the detrimental effect of agitation on bacterial viability found under specific experimental conditions (distilled water and low bacterial concentration) in solar photocatalytic reactors. The negative effects of water recirculation in solar reactors have not yet been described. Nevertheless, cell damage due to agitation has been studied in other processes [Cherry and Papoutsakis, 1986; Handa-Corrigan *et al.*, 1989; Zhang *et al.*, 1992; Wase *et al.*, 1985 a, b]. Due to the very complex interrelationships of inactivation mechanisms during disinfection, factors not forming part of solar and solar photocatalytic inactivation mechanisms that coexist in the process are often underestimated. Since these parameters have been demonstrated to significantly affect disinfection results under certain conditions, correct evaluation of photocatalytic performance can be hindered. This clearly affects reactor efficiency evaluation and therefore subsequent optimisation of the disinfection system. In addition to identifying and measuring such parameters coexisting in the solar photocatalytic process, this work aims to set criteria for solar reactor operation protocols during optimization.

Experimental methods

Solar CPC photoreactors: All the photocatalytic experiments were carried out under sunlight at the Plataforma Solar de Almería (Spain, local latitude 37º N, longitude 2.4º W) using compound parabolic solar collectors (CPC) fabricated by AOSOL Ltd. (Portugal) and installed in the experimental prototype manufactured by Ecosystem, Environmental Services, S.A. (Barcelona, Spain). All experiments were done in the morning on completely sunny days from May to July 2005.

The photoreactor module used was designed and built expressly for a photocatalyst immobilized on tubular supports. The supports are inserted in two borosilicate glass tubes (Glass Type 3.3, Schott-Duran, Germany, cut-off at 280 nm) which are placed in the focus of CPC reflectors (**Figure 3.1**) designed for the best optical performance under these particular conditions [Collares-Pereira, 2006]. The systems (tubes + supports + CPC

collectors) are held by aluminum frames mounted on platforms tilted 37° local latitude (**Figure 3.2**).

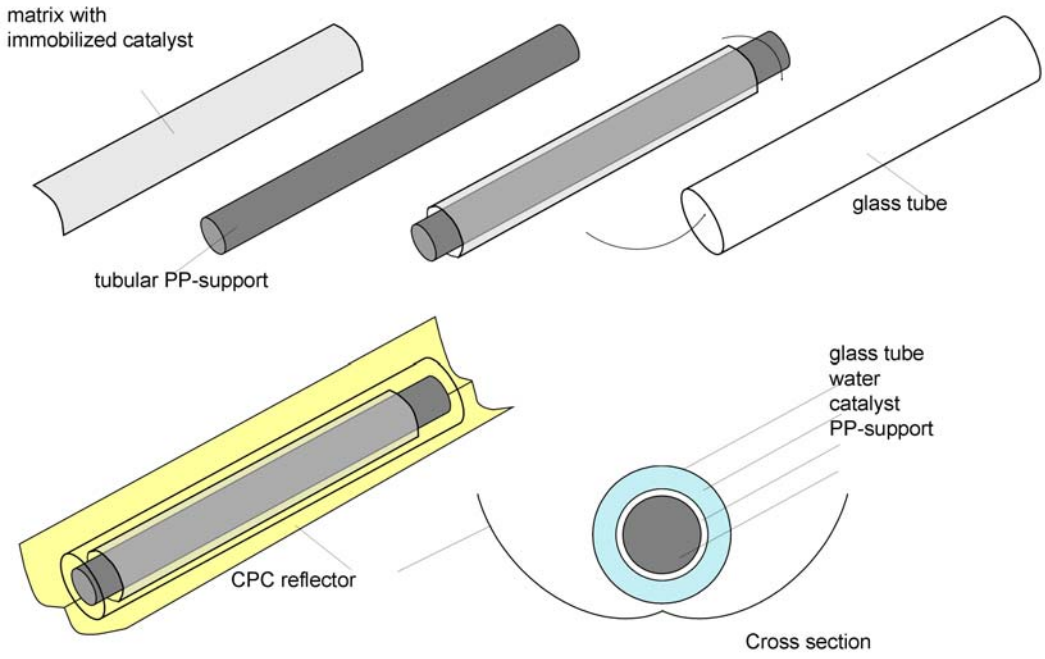

Figure 3.1 Diagram of the catalyst arrangement in the CPC reactor.

The glass tubes are connected so that water flows directly from one to another and finally into a tank (**Figure 3.2**). A centrifugal pump (20 Watts, Panworld, Spain) then returns the water to the collectors. The tank has an aperture on top where the contaminated water can be poured in. For the disinfection process, this aperture is closed with a plastic lid. The treated water is later recovered by opening the outlet valve. Similar CPC photoreactors have previously been described in detail elsewhere [Hermann *et al*., 2005; Blanco *et al*. 2000; Malato *et al*., 2002 a, b; Fernández *et al*., 2005]. The photoreactor volume is 14 L and the illuminated volume for the whole system is 2.27 L. The illuminated surface of the solar collector is 0.41 m^2. The outer diameter of the glass tubes is 50 mm.

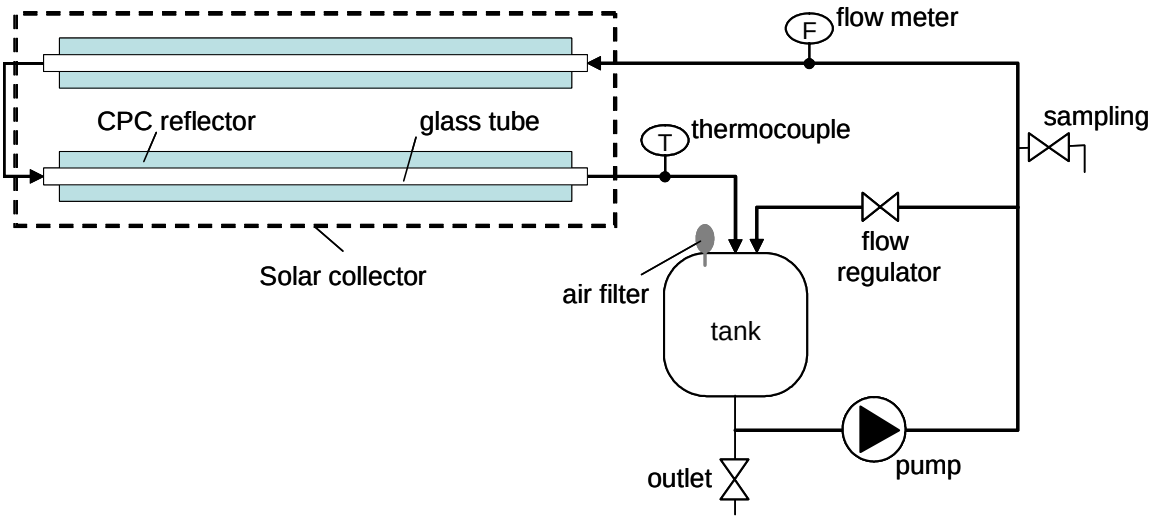

Figure 3.2 Flow diagram of the solar CPC photoreactor.

Catalyst: Fixed-catalyst experiments were performed using a synthetic fiber support, Type KN "Ahlstrom paper"© (organic fibers, homogeneous weave, 460-μm thick and weight 80 g/m2) manufactured by Ahlstrom Research & Services, France [Ahlstrom, 1999], and coated with Degussa P25 TiO_2 at a dose of 20 g TiO_2 /m^2 using an inorganic binder, an aqueous dispersion of colloidal SiO_2, which is transparent to UV radiation [Guillard *et al*., 2003; Ahlstrom, 1999]. Sheets of the coated Ahlstrom paper were fastened on the concentric support and inserted in the CPC photo-reactor for photocatalytic experiments (**Figure 3.1**).

The Ahlstrom catalyst was washed several times following a specifically designed protocol (three runs with only distilled water) before its first use to rule out the possibility of material leaching into the water. No TiO_2 was detected in spectrometric measurements of the rinse water.

Evaluation of solar UV radiation: Q_{UV} is used to interpret results in solar reactor systems. This magnitude estimates accumulated UV energy in the photoreactor per unit of treated water volume for given periods of time during the experiment. It is used to normalize the energy dose for the photocatalytic reaction in the CPC reactor.

The intensity of solar UV radiation is measured by a global UV radiometer (Mod. CUV3, KIPP&ZONEN, the Netherlands) with a typical sensitivity of 264 μV/W per m^2 and a central wavelength of 300-400 nm, mounted on a platform tilted 37º (the same angle as the CPCs), which provides data in terms of incident W_{UV}/m^2. With this, the total UV energy received on any surface in the same position with regard to the sun is calculated per unit of volume in the reactor using **Equation 1** [Kositzi *et al*., 2004] where t_n is the experimental time for n-sample, $\overline{UV}_{n-1}$ is the average solar ultraviolet radiation measured during the period $(t_n\text{-}t_{n-1})$, A_r is the illuminated collector surface and V_t, the total reactor volume.

$$Q_{UV} = \sum_n \overline{UV}_{n-1} \frac{A_r}{V_t}\left(t_n - t_{n-1}\right) \qquad \textbf{(Eq. 3.1)}$$

Consequently, when Q_{UV} is used, the reaction rate is expressed in terms of decrease in CFU (colony forming units) concentration per Jules of UV energy reaching the collector surface.

Bacterial strain and quantification: *E. coli* K-12, ATCC 23631 was inoculated in a Luria Broth nutrient medium (Miller's LB Broth, Sigma-Aldrich, USA) and incubated at 37 ºC with constant agitation on a rotary shaker at 100 rpm for 24 h. The stationary phase of bacterial growth yielded a concentration of 10^9 CFU/mL. For all experiments, the range of initial concentrations (C_0) of *E. coli* was from 10^4 CFU/mL to 10^7 CFU/mL.

For initial concentrations of 10^4 CFU/mL, *E. coli* suspensions were prepared with distilled water directly in the photoreactor tank by inoculating 140 μL of a concentrated culture in 14 L of water. For higher concentrations, *E. coli* suspensions were centrifuged at 3000 rpm for 10 min and washed three times with saline solution (0.9 % NaCl). Finally, the bacteria pellet was resuspended in distilled water and diluted in the 14-L tank to reach the required cell density. Samples were serially diluted in distilled water and plated. Every sample was plated 16 times (16 x 10 μL) on Luria agar (Sigma-Aldrich, USA). The detection limit for this method of quantification is 6 CFU/mL. Inoculated samples were incubated at 37 ºC for 24 h before counting.

CPC reactor experiments: "Dark runs" were performed under exactly the same photoreactor conditions as "solar TiO_2 photocatalysis" tests, but in the dark, by placing a black cover over the solar collectors. "Solar disinfection" experiments were carried out in the presence of sunlight without a catalyst, but with the catalyst support in the collector tubes. The bacteria suspension was prepared directly in the reactor using various *E. coli* concentrations. For homogenisation and to let bacteria adjust to the environment before exposure, the reactor was kept running in the dark for 15 min. The 0-min "control sample" was kept in the dark in a 15-mL tube at 20 ºC and stirred slowly (100 r.p.m.). After 90 min, this sample was re-plated at the same time as the 90-min reactor sample. For "saline solution" experiments, NaCl was added to the reactor water at 0.9% wt. All the other experiments were done with distilled water. Although the temperature of the water in the reactor was not controlled, this parameter was monitored during all the experiments to avoid testing at over 36 ºC. All experiments were repeated three times to ensure reproducibility of results. The results reported are the average of these three replicates. The error bars correspond to the statistical error of the 3 (replicates) x 16 (inoculations), i.e., a 95% confidence level.

Modelling with GinaFiT: The results from the photocatalytic experiments done with different initial bacterial concentrations were fitted to the Geeraed and Van Impe inactivation model, GinaFiT [Geeraed *et al*., 2000 and 2005]. This model has been employed successfully to fit solar disinfection inactivation results by Berney *et al.* [Berney *et al*., 2006 a, b]. It admits six different types of microbial survival tests: log-linear regression [Bigelow and Esty 1920], log-linear + tail [Geeraerd *et al.*, 2000], log-linear + shoulder [Geeraerd *et al*., 2000], log-linear + shoulder + tail [Geeraerd *et al*., 2000], Weibull model [Mafart *et al*., 2002], biphasic model [Cerf and Metro, 1977], and biphasic + shoulder model [Geeraerd *et al*., 2005]. For each inactivation curve modelled, the fit result was the smallest Root Mean Sum of Squared Errors (RSME). The RSME is considered by Geeraerd [Geeraerd *et al*., 2005] to be the most informative measure of the goodness of fit. Most of our results fit the log-linear regression + shoulder best. The Q_{90} value was also used for comparison of inactivation curves; this parameter represents the accumulated UV energy (Q_{UV}) necessary to reduce the concentration of viable bacteria by 90 %. Q_{90} was calculated on the basis of the GinaFiT model results.

Results and Discussion

Solar photocatalysis at high bacterial concentrations: In spite of the fact that bacterial viability decreases slightly in the absence of ions, solar and solar photocatalytic experiments were done with distilled water to avoid interference of specific ions and organic compounds with the photocatalytic process. Since, as shown below, the osmotic effect is less important in high bacterial concentrations, the photocatalytic experiments were done at initial concentrations over 10^5 CFU/mL. Above this concentration bacteria are still inactivated in the dark, but the photocatalytic result is not disturbed. The flow rate was 2 L/min for the experiments shown in **Figure 3.3**.

Bacterial concentration in the dark ("dark run") are observed to remain almost stable throughout the experiment, while solar disinfection led to a 3-log decrease in concentration and the solar photocatalysis experiment 6-log. Both inactivation curves show a shoulder effect that can be seen in all the photocatalytic disinfection curves presented. Average UV irradiation received during the TiO_2 disinfection experiment was 22 W/m^2 for a solar dose of 119.5 kJ/m^2.

At high bacterial concentrations, photocatalytic disinfection was therefore clear. This 6-log disinfection after 90 min of solar photocatalytic treatment is very promising for fixed TiO_2 catalyst applications. Similar results have been found previously, but only in the laboratory [Rincón and Pulgarin, 2003] or in small scale bottle reactors [Gelover *et al*., 2006]. Rincón and Pulgarin reported a 5-log disinfection after 120 to 150 min exposure to irradiation with different-strength TiO_2 fixed catalysts using milli-Q water in bottle reactors (40 mL) and a solar simulator (400 W/m^2). In photocatalytic experiments under natural sunlight, disinfection of 11 L in CPC reactors with supported catalysts was only 2-log in 60 min [Fernández *et al.*, 2005].

The interest in using the Ahlstrom, or any other immobilised catalyst, is development of a solar photocatalytic disinfection system feasible for water treatment. The main advantage of using an immobilised catalyst is that it avoids the need to separate fine TiO_2 particles from the suspension after treatment.

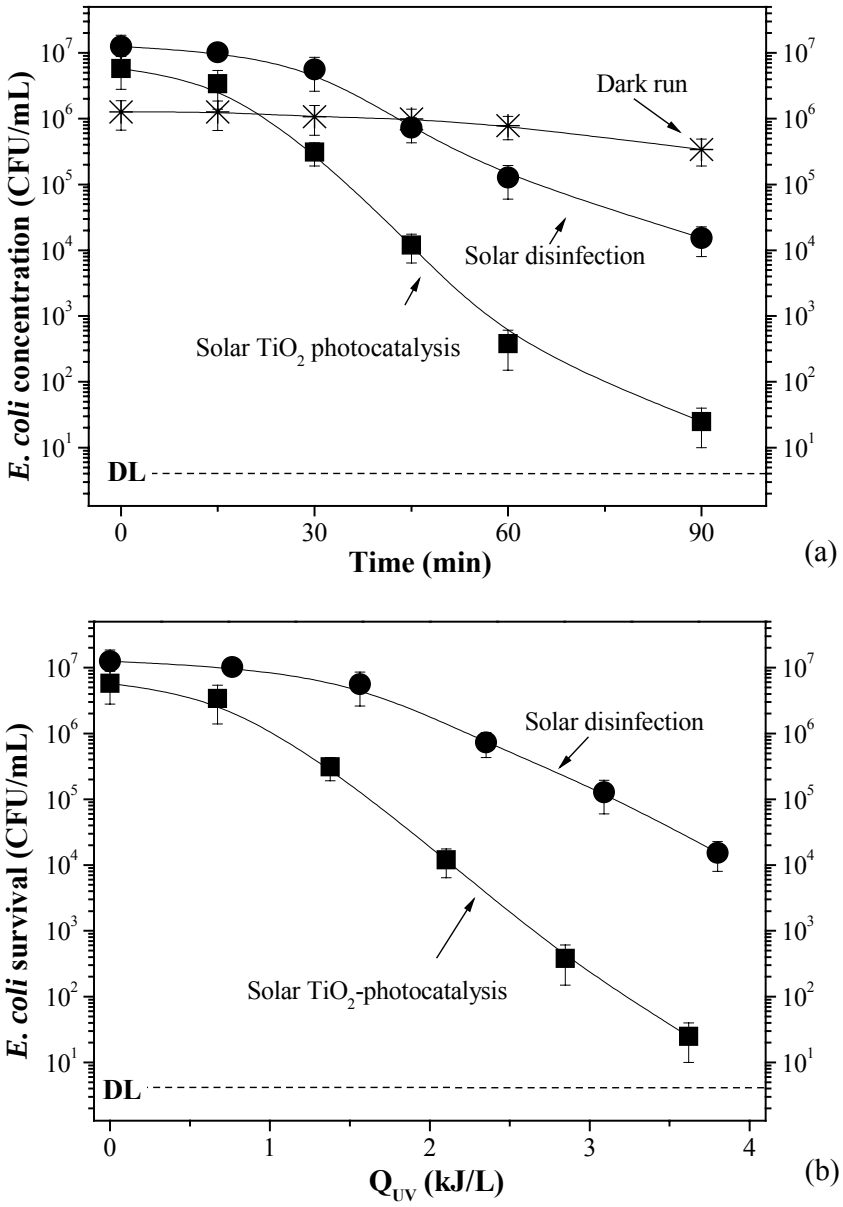

Figure 3.3 (a) *E. coli* concentration in the CPC reactor during solar disinfection (–●–) and solar TiO_2 photocatalytic experiments (–■–) at an initial bacterial concentration of around 10^7 CFU/mL. Dark run at C_0 ~ 10^6 CFU/mL (–×–). Distilled water flow rate 2 L/min. (b) *E. coli* concentration versus Q_{UV} for the same experiments, same symbols. Each point represents the average. The bars show the statistical error at a 95% level of confidence.

Mechanical stress superimposed on photocatalytic effects: The series of experiments shown in **Figure 3.4** compares experiments in the dark with experiments under solar radiation. Initial bacterial concentrations for these experiments were about 10^4 CFU/mL. An almost identical decrease in *E. coli* viability within the experimental error was observed

in both runs. In both cases, the bacterial concentration reached the detection limit after 90 minutes. Any possible adsorption of bacterial cells on the catalyst surface has been dismissed, because identical results were found during the "dark runs" without catalyst paper. These results show the strong impact that mechanical stress in the CPC reactors has on the viability of *E. coli* cells. Therefore, the effect of stress prevailing in the CPC reactor in the dark may be said to overlap with photocatalytic disinfection.

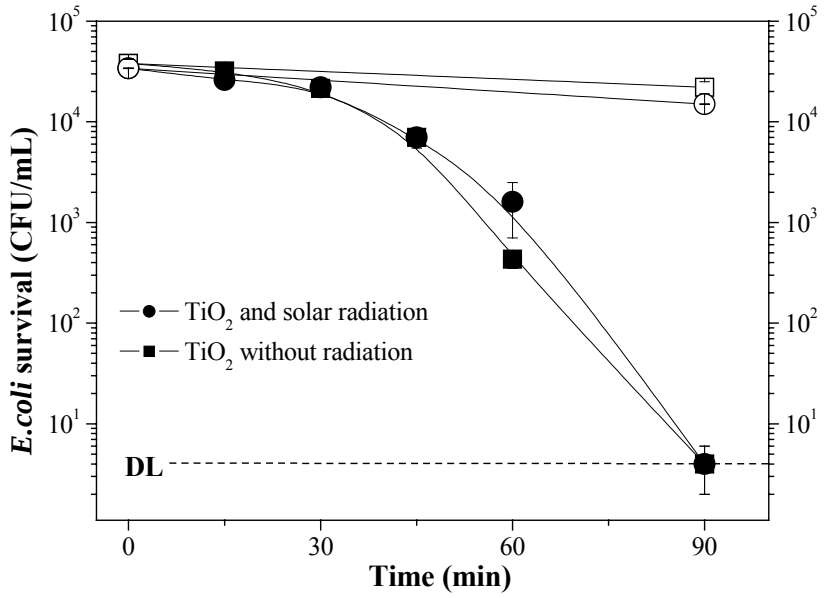

Figure 3.4 *E. coli* concentration in the CPC reactor in the dark (–■–) and under solar radiation (–●–). Initial bacterial concentration $3 \cdot 10^4$ CFU/mL. Distilled water flow rate 10 L/min. Open symbols (–□–, –○–) are control samples. Each point represents an average; the bars show the statistical error at a 95% level of confidence.

Influence of flow rate on photocatalytic disinfection: To study the effect of different flow rates on bacterial inactivation, a series of experiments was performed at 2, 5 and 10 L/min with the photocatalyst under sunlight (**Figure 3.5**) and in the dark (**Figure 3.6**). The best fits for the inactivation curves in **Figure 3.5** were obtained with GinaFiT for a log linear regression with shoulder. While the maximum reaction constants k_{max} found in the fit are very similar ($k_{2L/min} = 4.08$, $k_{5L/min} = 4.29$, $k_{10L/min} = 4.2$), the shoulder, and therefore, Q_{90}, increases with increased flow rate. At 2 L/min Q_{90} is 0.72 kJ/L, at 5 L/min 0.98 kJ/L, and for 10 L/min 1.52 kJ/L. This increase in the shoulder, or lag, means that photocatalytic disinfection is less at higher flow rates than at lower.

While in a TiO_2 slurry dissolved oxygen can become a reaction constraint [Rincón and Pulgarin, 2005], higher flow rates in the reactor lead us to expect better disinfection efficiencies, especially when oxygen only enters by contact with the air from water recirculation. This was confirmed in our work on TiO_2 in slurry at flow rates of 5 to 22.5 L/min [Fernández *et al.*, 2005].

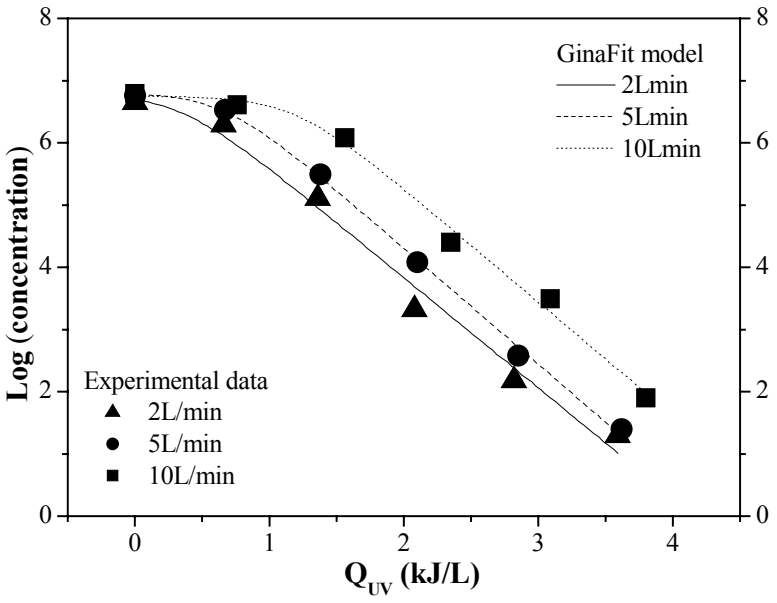

Figure 3.5 *E. coli* concentration in the CPC reactor during solar TiO_2 photocatalytic experiments in distilled water at 10 L/min (–■–), 5 L/min (–●–), and 2 L/min (–▲–). $C_0 \sim 5 \cdot 10^6$ CFU/mL. Corresponding GinaFit model fits: log linear regression with shoulder.

The main difference between fixed and slurry photocatalysis systems is the surface area of the catalyst, and therefore reaction yield and oxygen consumption are lower throughout the reaction. Consequently, it may be assumed that the main immobilised catalyst reaction constraint is the interaction between the catalyst surface and the target microorganism, not the oxygen supply. Agitation is not crucial for disinfection with fixed TiO_2 as shown by the very good disinfection results recently reported with fixed TiO_2 [Gelover *et al.*, 2006]. These authors demonstrated a 4-log decrease in fecal coliforms after 30 min irradiation in PET bottles with TiO_2 supported on glass cylinders by a sol-gel technique.

While **Figure 3.5** overlays dark and photocatalytic inactivation, **Figure 3.6** shows only inactivation in the dark. A slight impact on bacterial viability can be observed during the first 30 min of the experiment (0.5 log), but after 90 min at 10 L/min, dark inactivation contributes significantly (2 log) to total bacterial reduction. It can also be observed that dark inactivation increases with increasing flow rate. This tendency might be explained by "shear damage" to the bacterial cells from mechanical agitation. Although shear damage has not yet been completely described in solar reactors, the same tendency to bacterial survival in the dark was found by Fernández *et al.* [Fernández *et al.*, 2005].

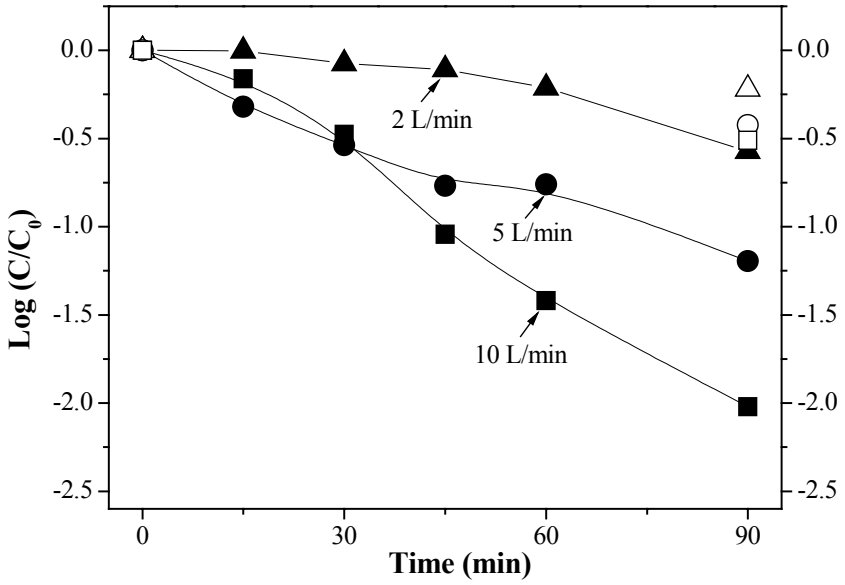

Figure 3.6 *E. coli* concentration during dark runs at flow rates of 10 L/min (–■–), 5 L/min (-●–), and 2 L/min (–▲–). $C_0 \sim 10^6$ CFU/mL. Open symbols (–□—○–, –Δ–) are control samples. Each point is an average; the bars show the statistical error at a 95% level of confidence

Shear damage to different types of cells is most often mentioned in bioprocesses [Thomas *et al*., 1990; Cherry and Papoutsakis, 1986; Handa, *et al*. 1989; Zhang *et al*. 1992]. There is even literature about shear damage to *E. coli* that reports increasing bacterial inactivation or changes in bacterial physiology with stronger mechanical agitation [Wase *et al.*1985 b, and Hewitt *et al.*, 1998]. Bacterial inactivation over the experimental time shows that bacterial resistance weakens with continued mechanical stress.

Opposite tendencies occurring during solar photocatalytic disinfection and in the dark can be explained by the different way in which bacterial viability is affected by mechanical stress (dark) or attack by radicals (sunlight+TiO_2). While the mechanical stress slowly but continuously gets stronger with higher flow rates over experimental time, radicals overwhelm bacterial resistance only after a lag phase, which is shorter at slow flow rates, due to better interaction between bacterial cells and photocatalyst.

Influence of initial bacterial concentration on bacterial inactivation: **Figures 3.7** and **3.8** show experiments with various initial concentrations at 10 L/min to determine the influence of the initial bacterial concentration on the photocatalytic disinfection and the bacterial viability in the dark. During solar photocatalytic disinfection (**Figure 3.7**), the experiment starting at 10^5 CFU/mL had a final concentration of 10 CFU/mL, at 4 kJ/L, while the 10^6 CFU/mL experiment decreased to 20 CFU/mL at 3.6 kJ/L and the 10^7 CFU/mL experiment to 90 CFU/mL at 4.2 kJ/L.

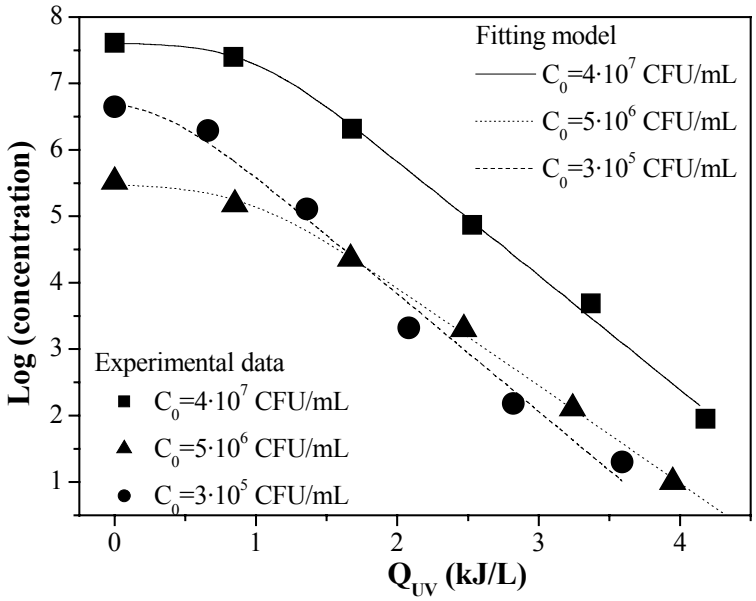

Figure 3.7 *E. coli* log concentration in the CPC reactor during solar TiO_2 photocatalytic experiments in distilled water at 2 L/min flowrate for three initial bacterial concentrations, $4 \cdot 10^7$ CFU/mL (–■–), $5 \cdot 10^6$ CFU/mL (–●–) , and $3 \cdot 10^5$ CFU/mL (–▲–).

While there is little difference between the lower concentrations of 10^5 and 10^6 CFU/mL, at the highest concentration of 10^7 CFU/mL, more UV energy is needed for the same

disinfection. This tendency to first-order kinetics has been reported previously for disinfection with TiO_2 slurries [Rincón *et al*., 2004 b; Rincón *et al.*2003]. However, when fitted to a logarithmic scale, the disinfection rate is not linear, but has a shoulder at the beginning.

Figure 3.7 shows the best fits found by GInaFiT for a log linear regression with shoulder, which has also been reported for most solar bacteria disinfection processes. Its shape depends on the strain and the specific growth rate of bacteria [Berney *et al.,* 2006 a; Berney *et al.,* 2006 b; Rincón *et al.,* 2003]. In the first step of the solar disinfection treatment, the shoulder effect can be attributed to UV-induced self-defence mechanisms [Rincón *et al.,* 2003]. Our results with the catalyst show a smaller shoulder than those found for solar disinfection in the studies mentioned. The kinetics observed here can be explained by simultaneous solar TiO_2 and solar-only disinfection. The fixed catalyst improves the action of sunlight alone, as shown in **Figure 3.3**, but does not reach the fast first-order inactivation reported for TiO_2 slurry.

For the dark studies (**Figure 3.8**) the experiment performed at an initial bacterial concentration of 106 CFU/mL shows a 2-log decrease in viability (99%), the one at 10^5 CFU/mL, a 1-log decrease (95%), and the one at 10^4 CFU/mL, a 4-log decrease (99.96%). In our experiments the 10^4-CFU/mL initial concentration was critical, as the detection limit was reached within 90 min. Higher bacterial concentrations made the influence of dark inactivation less noticeable in the final disinfection results.

Influence of bacterial concentration on its viability in different types of water has also been reported by Kerr *et al*., who studied *E. coli* viability in distilled water, sterile mineral water and in natural mineral water. Water quality turned out to be less important for bacterial survival at higher bacterial concentrations than at lower [Kerr *et al*., 1999]. Tailing in the curves in **Figure 3.8** might be caused by the presence of a part of the bacterial population being more resistant to imposed osmotic and mechanical stress. Such tailing is also observed in disinfection processes, where resistant populations dominate the inactivation rates [Rincón *et al.,* 2004 b].

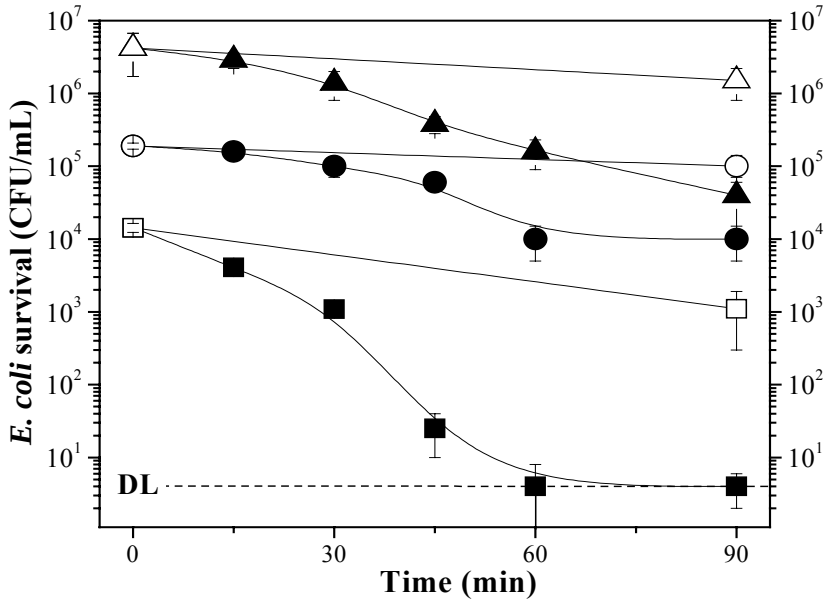

Figure 3.8 *E. coli* concentrations in the CPC reactor during three dark runs at various initial bacterial concentrations: 10^4 CFU/mL (–■–), $2{\cdot}10^5$ CFU/mL (–●–), $4{\cdot}10^6$ CFU/mL (–▲–). Open symbols (–□–, –○–, –Δ–) represent control samples. Each point represents the average value; the bars show the statistical error at a 95% level of confidence.

Influence of osmotic stress on bacterial viability: To determine any possible effect of osmotic stress on bacterial cells, experiments were performed in the dark with distilled water and a saline solution (0.9 % wt. NaCl) at 2 L/min, because the least mechanical stress was expected at this flow rate. The bacterial concentration most affected, 10^4 CFU/mL, was chosen as the initial concentration for this experimental series (**Figure 3.9**).

While the bacteria in distilled water decreased 4 log to the detection limit, the concentration in the saline solution decreased less than one log (0.7 log). Such different behaviour shows the effect of mechanical stress with and without osmotic stress. On the other hand, the bacterial concentration in the saline control sample remained stable during the experiment in the dark, while the distilled water control sample showed a 0.4-log decrease in concentration, the same as in all the other experiments performed in distilled water, demonstrating the effect of osmotic stress without any other influence. Thus

mechanical stress alone together with osmotic stress alone would only explain a decrease in viability of approx. 1 log. The fact that our results in distilled water in the dark show significantly higher bacterial inactivation than additional osmotic and mechanical stress alone, can be explained by the influence of mechanical agitation on the bacterial cell osmoregulatory system.

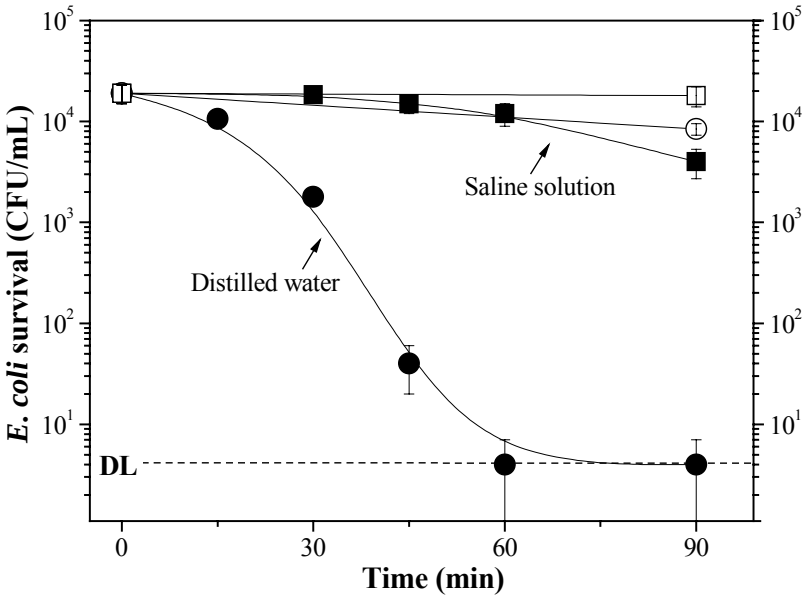

Figure 3.9 *E. coli* concentration in the CPC reactor during two dark runs with distilled water (–●–) and saline solution (–■–). $C_0 \sim 10^4$ CFU/mL. Flow rate 2 L/min. Open symbols (–□–, –○–) represent the control samples. Each point is an average. The bars show the statistical error at a 95% level of confidence.

It is commonly accepted that *E. coli* osmolarity, whether osmolarity conditions are high or low, is regulated through mechanosensitive channels [Blount *et al.,* 1996; Levina *et al.,* 1999; Booth and Louis, 1999; Biggin and Sansom, 2003; Calamita *et al.*, 1998]. These mechanosensitive channels permit bacteria to maintain turgor pressure even under severe changes in osmolarity. In low osmolarity environments, water starts entering the cell due to osmotic pressure, mechanosensitive channels expel ions into the media and turgor pressure consequently decreases. Wase *et al.* found a linear increase in cell volume with increasing agitation rates for chemostat-cultivated *E. coli*, which they explain by an increase in water content in the cells [Wase *et al*., 1985 a].

In a later paper they reported a sharp increase in intracellular ion concentration in *E. coli* as agitation rates increased [Wase *et al*., 1985 b]. These authors give several possible explanations for this, but the reason for the increase in measured ion concentration is not completely clear. Nevertheless, these contributions lead us to expect that mechanical agitation under certain conditions affects the *E. coli* osmoregulatory system. We therefore think that in our case, mechanical stress due to recirculation in the reactors may interfere with the complex osmoregulation of the bacterial cell in distilled water, and therefore causes changes in bacterial resistance during the disinfection process.

Influence of temperature: Temperature was monitored during all experiments. The temperature profiles of the water in the photoreactor during photocatalytic and dark viability experiments are shown in **Figure 3.10**. The temperature curves shown are the highest and lowest profiles during the experiment series. The highest temperatures were measured in disinfection experiments done in July.

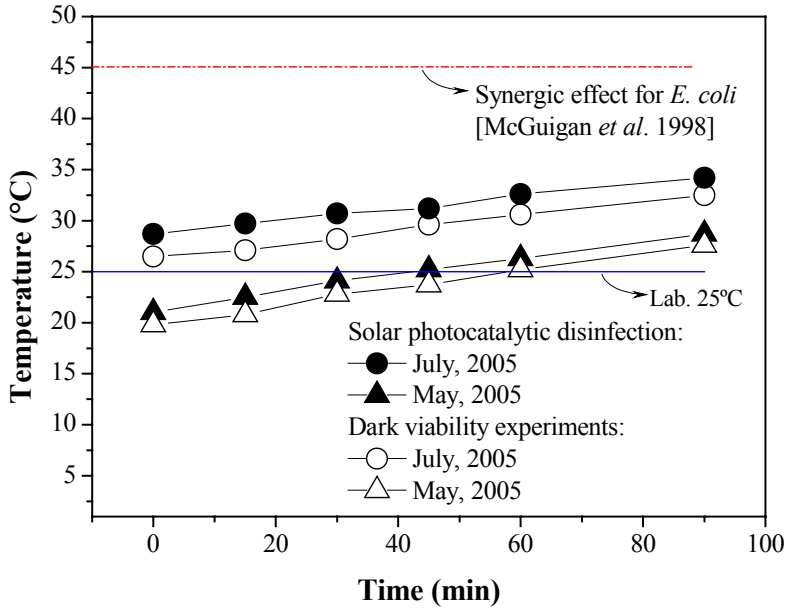

Figure 3.10 Temperature in the CPC reactor during solar photocatalytic (–●–, –▲–) and dark control experiments (–○–, –Δ–). Control samples were stored at in the lab 25 ºC in the dark (—).

This curve shows slightly higher temperatures (approx. 2 ºC) than in the dark viability experiments also done in July. The lowest temperatures were found in disinfection and

dark experiments done in May. Even the experiments in July did not reach 40 ºC, the temperature required for synergy of solar irradiation and temperature [McGuigan *et al.,* 1998].

Temperatures were between 19.8 ºC and 32.5ºC in all viability experiments, so that the decrease in bacterial concentration cannot be due to a thermal effect. Nevertheless, increasing cell metabolic activity might increase the response to osmotic stress, and thereby also contribute to increasing bacterial inactivation during the experiment.

Conclusions

- Using TiO_2 immobilized on Ahlstrom paper at concentrations of 10^6 ^{c}FU/mL, photocatalytic disinfection was complete after 90 min in the CPC reactor. The Ahlstrom catalyst thus reduces bacterial concentrations significantly faster than solar disinfection alone.

- Photocatalytic disinfection was found to be more efficient at lower flow rates. This means that low-power pumps should be used for these applications. Such reduced energy consumption is of special interest for rural water disinfection systems operating on a solar power supply.

- Depending on experimental conditions, approximately 99 % of bacterial inactivation was shown to be caused by this mechano-osmotic dark inactivation during experiments in solar reactors. Mechanical inactivation increased with rising flow rates and was notably reduced in saline solution (water with NaCl 0.9 % wt.). This phenomenon is of great importance when evaluating the performance of solar photocatalytic disinfection systems (a solar reactor and photocatalyst). Photocatalytic disinfection of distilled water at bacterial concentrations below 10^5 CFU/mL is caused entirely by mechano-osmotic effects, which, if not taken into account, could be attributed to solar photocatalysis.

Acknowledgements

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IV.4. Effect of UV- intensity and dose on the photocatalytic disinfection of bacteria and fungi

Abstract

The purpose of this work was to study the dependence of solar photocatalytic and solar water disinfection on solar irradiation conditions under natural sunlight. This dependency was evaluated for solar photocatalysis with TiO_2 and solar-only disinfection of three microorganisms, a pure *E. coli* K-12 culture and two wild strains of the *Fusarium* genus, *F. solani* and *F. anthophilum*. Photocatalytic disinfection experiments were carried out with TiO_2 supported on a paper matrix around concentric tubes, in compound parabolic collectors (CPCs) or with TiO_2 as slurry in bottle reactors, under natural solar irradiation at the Plataforma Solar of Almería (Spain). The experiments were performed with different illuminated reactor surfaces, in different seasons of the year, and under changing weather conditions (i.e., cloudy and sunny days). All results show that once the minimum solar dose has been received, the photocatalytic disinfection efficacy is not particularly enhanced by any further increase. The solar-only disinfection turned out to be more susceptible to changes in solar irradiation, and therefore, only took place at higher irradiation intensities.

Keywords: *E. coli*, *F. solani*, *F. anthophilum*, solar energy, photocatalytic disinfection, TiO_2.

Introduction

Water quality and its sustainable supply are major worldwide concerns. Standards and controls avoiding contamination by microorganisms, especially pathogenic specimens, underlie the safety of drinking water. Depending on the final use, water requirements, such as the WHO Guidelines for drinking water, may be very strict. Water used in agriculture must also comply with minimum safety standards, since irrigating water is a vehicle for plant pathogens and contagion, especially in intensive greenhouse agriculture, where the reuse of irrigation water is often limited due to contamination by phytopathogens. Water

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that does not meet these standards must be disinfected before its recirculation.

Because of its cheap, easy application, chlorination is often used for disinfection, however, among its negative effects are the appearance of trihalomethanes (THMs) as the by products of its reaction with organic matter, phytotoxicity, and an unpleasant taste when used for drinking water [Jarvis 1998, Metcalf and Eddy, 2005]. Both the thermal energy of sunlight and its germicide properties have recently been used for disinfection. [McGuigan *et al.,* 1998, 2006]. In addition to large-scale applications in solar reactors [McLoughlin *et al.,* 2004, Fernandez *et al.,* 2005; Sichel *et al.,* 2007 a], research for solar disinfection in recent years has often focused on small-volume (1.5L) UV-light-transparent plastic bottles as batch reactors for domestic treatment of drinking water in areas with high solar irradiance [McGuigan *et al.,* 1998, Joyce *et al.,* 1996]. Called the SODIS treatment, it has been proven successful for a wide range of microorganisms including bacteria, fungus spores, cysts etc. [McGuigan *et al.,* 2006 ; Berney *et al.,* 2006 ;a, b, c, Lonnen *et al.,* 2005].

Nevertheless, this type of solar disinfection is limited in applications with high volumes of water or more resistant microorganisms, and may therefore be enhanced by the addition of a photocatalyst semiconductor. This Advanced Oxidation Technology can be deployed for air and water treatment using TiO_2, an already well-analyzed, low-cost photocatalyst. The wide range of TiO_2 applications was recently reviewed by Fujishima [Fujishima *et al.,* 2000]. Among its other applications, TiO_2 for the treatment of different types of water contamination has been studied in depth [Herrmann *et al.,* 1998].

When TiO_2 is excited by the energy of near UV-radiation (<400 nm) a photocatalytic reaction that generates electron-hole pairs on the semiconductor surface takes place [Herrmann 2005]. In the presence of water and oxygen, hydroxyl radicals ($^{\bullet}OH$) are formed [Hoffmann *et al.,* 1995]. Hydroxyl radicals are known to be strong, not very selective oxidizing agents. Furthermore, water treatment with TiO_2 photocatalysis does not require the addition of consumable chemicals. Killing microbial cells with a photoexcited semiconductor powder was first reported by Matsunaga and co-workers in 1985 [Matsunaga *et al.,* 1985]. Since then, an increasing amount of research has contributed to the development of new materials, supported photocatalysts, photoreactors and procedures

for water disinfection. Results suggest that oxidative damage first takes place on the cell wall, where the TiO_2 photocatalytic surface makes contact with the cell [Maness *et al.,* 1999; Sunada *et al.,* 2003].

Maness presented evidence of peroxidation of the polyunsaturated phospholipid component of the lipid cell membrane, which produces major life-threatening damage leading to a loss of essential functions, such as respiratory activity, and consequently to cell death [Maness *et al.,* 1999]. Although it is uncertain what reactive oxygen species are directly involved in the photokilling process, the main reactive oxygen species (ROS) are hydroxyl radicals [Cho *et al.,* 2004], $O_2^{\bullet-}$ and H_2O_2 [Rincón and Pulgarin, 2005]. Many publications have focused on the application of TiO_2 photocatalytic disinfection at laboratory scale [Blake *et al.,* 1999], however those concentrating on the use of solar energy [Zhang *et al.,* 1994; Block *et al.,* 1997], or on pilot-plant scale applications under natural sunlight are scarce [Fernandez *et al.,* 2005; Sichel *et al.,* 2007 a; Vidal *et al.,*1999; Vidal and Díaz 2000; Rincón and Pulgarin, 2004 a]. Some work has also been published on the successful disinfection of water from real sources such as rivers, lakes and wells [Rincón and Pulgarin, 2004 b; Wist *et al.,* 2002].

Disinfection yield with chemicals depends on the concentration of the bactericidal agent and contact time with the microorganisms [Bitton, 2005]. In UV-disinfection systems, the yield depends on UV-lamp irradiation time (254 nm), characterized by their performance [Masschelin, 2002]. The most common way of comparing the resistance of different types of microorganisms to a UV-disinfection system is by determining the "UV dose" in kJ /m^2, defined as the product of the UV intensity, expressed as UV irradiance (W/m^2) [Perry, 1992], and irradiation time needed for a given disinfection level, as measured by the decrease in colony forming units (CFU) [Gill and McLoughlin 2007; Rincón and Pulgarin, 2005; Lonnen *et al.*, 2005; Berney *et al.,* 2006 a, b].

The efficacy of solar systems, in which the sun is the source of photons at a wavelength of 300 to approx. 1400 nm [ASTM, 1987], can also be characterized by the disinfecting "solar UV dose" (kJ /m^2). In solar reactor systems, the unit considered is often the accumulated solar UV energy (kJ L^{-1}) received during photocatalytic disinfection [Rincón and Pulgarin,

2004 a; Herrmann, 2005; Fernandez *et al.,* 2005]. Some authors consider the global solar spectrum when comparing natural and simulated sunlight disinfection performance [McGuigan *et al.,* 2006].

Nevertheless, the most suitable way of comparing solar disinfection system efficiency (with or without catalyst) still remains undecided. This is probably due to the changeable irradiation conditions of sunlight and the limited knowledge of the mechanisms governing the solar-only and solar photocatalytic (TiO_2) disinfection processes. Even if only the definition of specific criteria is considered essential, very few contributions have focused on the issue of comparing solar photocatalytic disinfection experiments by the solar dose or solar energy received, as do Rincón *et. al* [Rincón and Pulgarin, 2004 b].

The goal of this work was to determine disinfection performance by the solar dose and energy per unit of volume received under very different experimental conditions and microorganisms. This was assessed in two reactor systems, a) a CPC solar reactor pilot plant with *E. coli*, b) bottle reactors with the fungi, *F. solani* and *F. anthophilum*. Three different types of solar radiation experiments were studied: i) at different times on sunny days close together in the same season and ii) in different seasons; and iii) solar tests done on days close together, but under different weather conditions (cloudy and sunny).

Experimental

Bacteria strain and quantification: E. coli K-12 was inoculated in a Luria broth nutrient medium (Miller's LB Broth, Sigma-Aldrich, USA) and incubated at 37 ºC by constant agitation under aerobic conditions. Growth was monitored by optical density measurement at 600 nm. Bacteria were collected after 24 h of stationary-phase incubation, yielding a concentration of 10^9 CFU/mL. *E. coli* suspensions were centrifuged at 3000 rpm for 10 min and washed three times with saline solution (0.9 % NaCl). Finally, the bacteria pellet was resuspended in distilled water and diluted in the 14-L photoreactor tank to the cell density required for the initial concentration. The reactor was kept running in darkness for 15 minutes to allow bacteria to adjust to the environment and come to a homogeneous suspension before exposure. Then a 0-min sample was taken and kept in the dark as a "control sample". After 90 min this "control sample" and the 90-min reactor sample were

simultaneously replated. The samples taken during the experiment were serially diluted in distilled water and plated 16 times (16x10 μL) on Luria agar (Sigma-Aldrich, USA). Colonies were counted after incubation for 24 h at 37 °C. Where fewer than 10 colonies per plate were observed, 250 μL of the sample were plated for a detection limit of 4 CFU/mL.

Fungal strain and quantification: The fungi used were *F. solani* and *F. anthophilum.* Both fungi were chosen for their importance as pathogenic fungi in agriculture and their medium (*F. solani*) and low (*F. anthophilum*) resistance to photocatalytic disinfection as found in previous work [Sichel *et al.,* 2007 b]. The strains were original wild fungi obtained from soil cultures in Almería (southern Spain). The fungal colonies were transferred to an agar sporulation medium containing sodium and potassium chloride in Petri dishes exposed to UV-C radiation from a mercury lamp (40 W) for 15 days at 25 ºC to produce the required number of spores (10^5-10^7 CFU/plate). [Sichel *et al.,* 2007 b].

Our disinfection work was based on spore inactivation as spores are the most resistant fungal life form. The spores were detached from the mycelia and agar by washing the plates with distilled water. The suspension obtained was homogenized by mechanical agitation and the concentration was determined by direct count using a phase contrast microscope. The initial adjusted experimental concentration was approx. 10^3 CFU/mL. 50-250-μL samples of the water were plated out using the spread-plate technique on acidified (pH4) malt agar (Sigma Aldrich, USA). Where fewer than 10 colonies per plate were observed, 500 μl of the sample were plated to a detection limit of 2 CFU/mL. Each measurement was made in triplicate, with three sample inoculations on three plates. After two days of incubation at 28 ºC in the dark, the colonies were counted. The same protocol had already been used in previous work [Sichel *et al.,* 2007 b].

Solar CPC reactor (pilot plant) and supported catalyst: The solar reactors used for bacteria disinfection were compound parabolic solar collectors (CPCs) developed and manufactured by AoSol Ltd. (Portugal) [Malato *et al.,* 2004]. Two borosilicate glass tubes with cylindrical polypropylene supports inside (32-mm diameter) were placed in the focus of the CPC reactor. The CPC modules (tubes, supports, and CPC) were mounted on a fixed

platform tilted 37° (local latitude) and connected in series so that water flowed directly from one to another and finally into a tank. A centrifugal pump returned the water to the collectors. The illuminated solar collector surface was 0.4 m^2. The outer diameter of the glass tubes was 50 mm and the illuminated volume of the whole system was 2.27 L out of a total reactor volume of 14 L. The CPC photoreactor system with the concentric support has been described in detail elsewhere [Sichel *et al.,* 2007 b]. The water temperature in the reactor was monitored during all the experiments and was always below 30 ºC. All experiments were performed in duplicate under natural sunlight using twin solar CPC reactors installed at the Plataforma Solar of Almería. All experiments started between 9:00 - 10:30 a.m. local time.

Bacteria disinfection experiments in the CPC solar reactors were made with immobilized TiO_2 manufactured by Ahlstrom Research & Services, France [Ahlstrom, 1999]. The 20 g-TiO_2 /m^2 catalyst load was immobilised on synthetic fibers called "Type KN Ahlstrom paper"© (activated carbon, uniformly woven, 460-μm thick, and grammage 80 g /m^2). The matrix was coated with Degussa P25 TiO_2 using an aqueous dispersion of colloidal SiO_2 binder transparent to UV radiation [Ahlstrom 1999; Guillard *et al.,* 2003]. The concentric supports were wrapped with sheets of Ahlstrom paper and then inserted in the glass tubes. After this, the solar reactor was filled with distilled water and covered to avoid light entering during bacteria inoculation in the reactor. Once the bacteria suspension had been circulated for 15 min (adaptation and homogenization time), the cover was removed and the experiment began.

Solar bottle reactor and slurry catalyst: The experiments with fungi were performed in 250 mL DURAN-Glass (Schott, Germany) reactor bottles. Glass covers (Schott) were used instead of plastic lids, to allow the solar radiation to enter the bottle reactor from all directions. The bottles were stirred with magnetic stirrers at 100 rpm during the experiment. All experiments were performed in triplicate under natural sunlight at the Plataforma Solar de Almería (latitude 37.09 ºN, longitude 2.36 ºW). Solar photocatalytic and solar-only disinfection tests were carried out simultaneously and the control bottles (one with and another without TiO_2) were stored under the same conditions, but in the dark for each *Fusarium* species. The temperature, measured during all experiments, was always

below 30 ºC, allowing thermal inactivation of fungi to be discarded, as *Fusarium* spores have been shown to still be germinating at this temperature [El Halouat and Debevere, 1997; Samapundo *et al.,* 2007]. Nevertheless, the dark control samples were kept at the same temperature as solar experiments to avoid thermal differences between irradiated and non-irradiated samples.

For the bottle reactor experiments, Degussa P25 TiO_2 was used as slurry at a concentration of 35 mg/L, which is the optimal catalyst load for the optical path in this reactor [Fernández-Ibáñez *et al.,* 1999]. The fungus suspension was added to the total 200-mL volume of water and left for 15 min in the dark (adaptation and homogenization time). Thereafter, the TiO_2 slurry catalyst was added and the bottle reactors were exposed to solar irradiation on a horizontal platform.

Solar radiation evaluation: UV radiation was measured with two similar global UV-A radiometers (Model CUV3, KIPP&ZONEN, the Netherlands, with a typical sensitivity of 264 μV W^{-1} /m^2), one of them mounted on a platform tilted 37º, and the other with no inclination. In this way, it was possible to evaluate the solar UV irradiance received in the two above mentioned solar systems, the tilted CPC reactor and the bottle reactor. For comparison of solar test results, the solar radiation was evaluated in terms of solar UV irradiance, which is defined as the rate at which solar radiant energy is incident on a surface per unit area of surface (W/m^2) [Duffie and Beckman 1991], solar UV dose received on the illuminated reactor surface, I_{UV} (J /m^2), and the accumulated UV energy per unit of treated water volume, Q_{UV} (J/L), which is frequently used for applications in solar reactors (**Equation 4.1**) [Fernandez *et al.*, 2005, Rincón and Pulgarin, 2004 b].

$$Q_{UV} = \sum_{n} \overline{UV}_{n-1} \frac{A_r}{V_t} \left(t_n - t_{n-1}\right) \qquad \textbf{(Eq. 4.1)}$$

where t_n is the experimental time for n-sample, $\overline{UV}_{n-1}$ is the average solar ultraviolet radiation measured during the period $(t_n\text{-}t_{n-1})$, A_r is the illuminated collector surface and V_t, the total reactor volume.

Results and Discussion

Effect of irradiated CPC solar reactor collector surface on E. coli disinfection: The effect of changing the illuminated reactor surface on solar photocatalytic disinfection was found in simultaneous experiments with different illuminated collector surfaces. The experiments were repeated on another day with similar light irradiance (intensity) under the same conditions.

Figure 4.1 a shows the progress of *E. coli* concentration in the CPC reactor during two experimental series with 0.4-m^2 and 0.2-m^2 irradiated collector surfaces. In both cases, a 4.5-log decrease in cultivable bacteria was observed after 90 min of photocatalytic treatment. In both experiments, the average solar UV irradiance was 16.5 W /m^2, therefore the solar UV dose received during the solar tests was 89 kJ /m^2. The parameter varied was the accumulated UV energy per unit of volume (Q_{UV}) entering the water in the reactor, which was 1.3 kJ/L and 2.6 kJ/L respectively.

Figure 4.1 a shows that *E. coli* was inactivated following the same kinetics in both experimental systems even though one of them received twice the solar UV energy as the other. **Figure 4.1 b** shows the results plotted against Q_{UV}, where it can be observed that the experiment in which the surface was irradiated less leads to faster bacteria inactivation for the UV energy per unit of volume received. This can be interpreted as the system with less illuminated area receiving fewer solar UV photons, achieves the same disinfection result, a 4.5-log decrease in *E. coli* after 90 min. This means that in the system with twice the irradiated surface, that is, twice the amount of incoming photons, photon efficiency is lower, probably because not all the photons are necessary for disinfection. It can therefore be concluded that when a solar photocatalytic system has received the UV energy necessary for disinfection, an increased reactor surface does not necessarily lead to better disinfection performance. Similar behaviour has been recently reported assuming that a minimum amount of energy is sufficient to inactivate all the bacteria cells [Berney *et al.,* 2006 a; Rincón and Pulgarin, 2004 b].

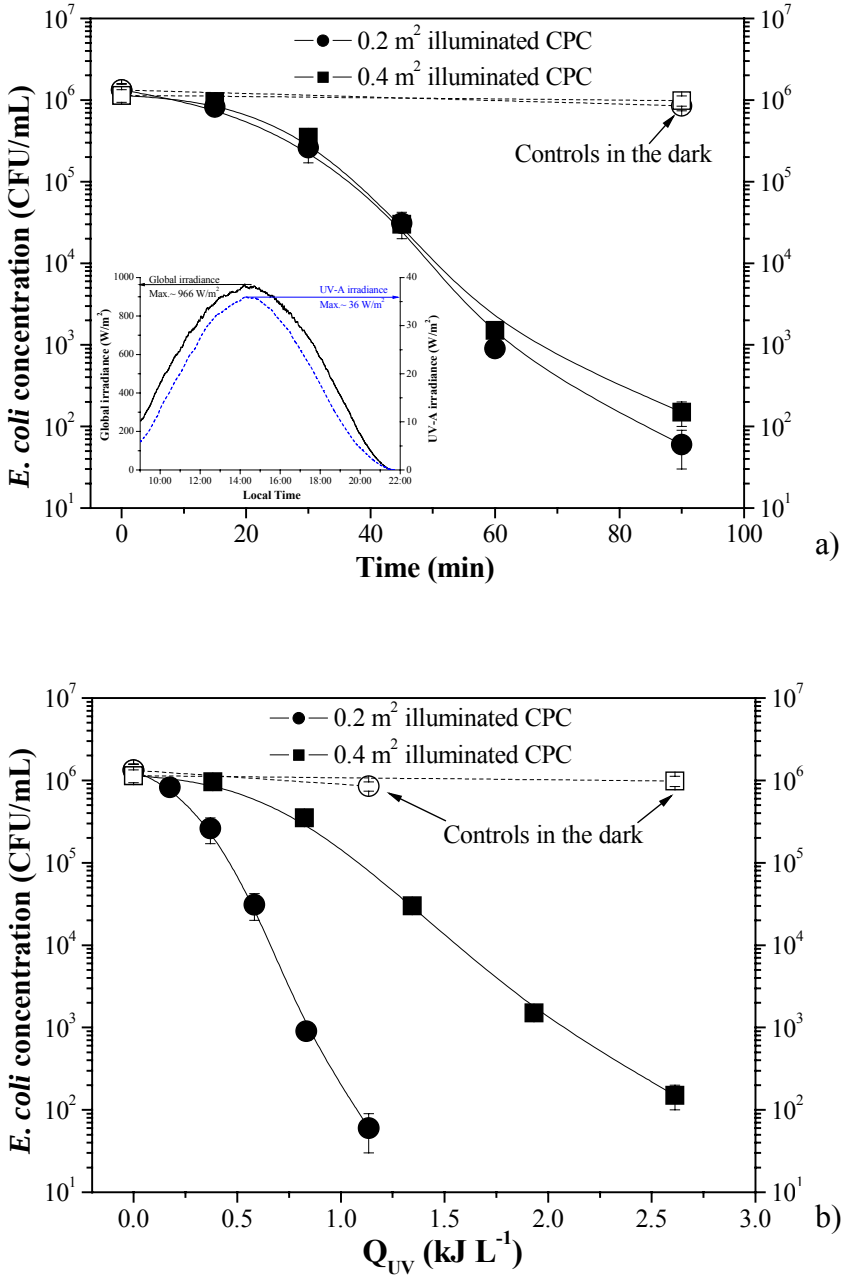

Figure 4.1 Solar inactivation of *E. coli* with the immobilized Ahlstrom catalyst in the CPC reactors with 0.2 m^2 (—●—) and 0.4 m^2 (—■—) of irradiated collector surface over time (a) and Q_{UV} (b). The inset contains the solar UV (---) and global (—) irradiance measured during the experiments (8 May, 2005, in Almería, Spain). Each point represents the average of replicates, and vertical lines show the statistical error at 95% confidence level.

Results from our previous work with solar-only disinfection confirm that *E. coli* inactivation in CPC reactors is not significantly improved by larger illuminated collector

surfaces [Fernandez *et al.,* 2005]. The results presented here confirm this tendency, even though the solar-only effect is combined with photocatalysis. Therefore, to the extent that the work in the CPC photoreactors represents an approximation to real water disinfection field applications, the possibility of saving in materials for CPC modules with a smaller reflector surface is of great interest.

E. coli inactivation in summer and winter: **Figures 4.2** and **4.3** show the seasonal influence on photocatalytic inactivation of *E. coli* in the CPC reactor. The experimental series is plotted as the initial (solid grey bars) and final bacteria concentrations (striped bars) over Q_{UV}, as found in several experiments in the CPC reactor with 0.4 m^2 of irradiated surface for 90 min on completely sunny mornings. **Figure 4.2** shows spring and summer disinfection performance, from the 1st of March until the 1st of October, 2004 and 2005, under strong solar irradiation. Q_{UV} was from 4 to 13 kJ L^{-1} and yielded an average 5-log reduction in cultivable bacteria.

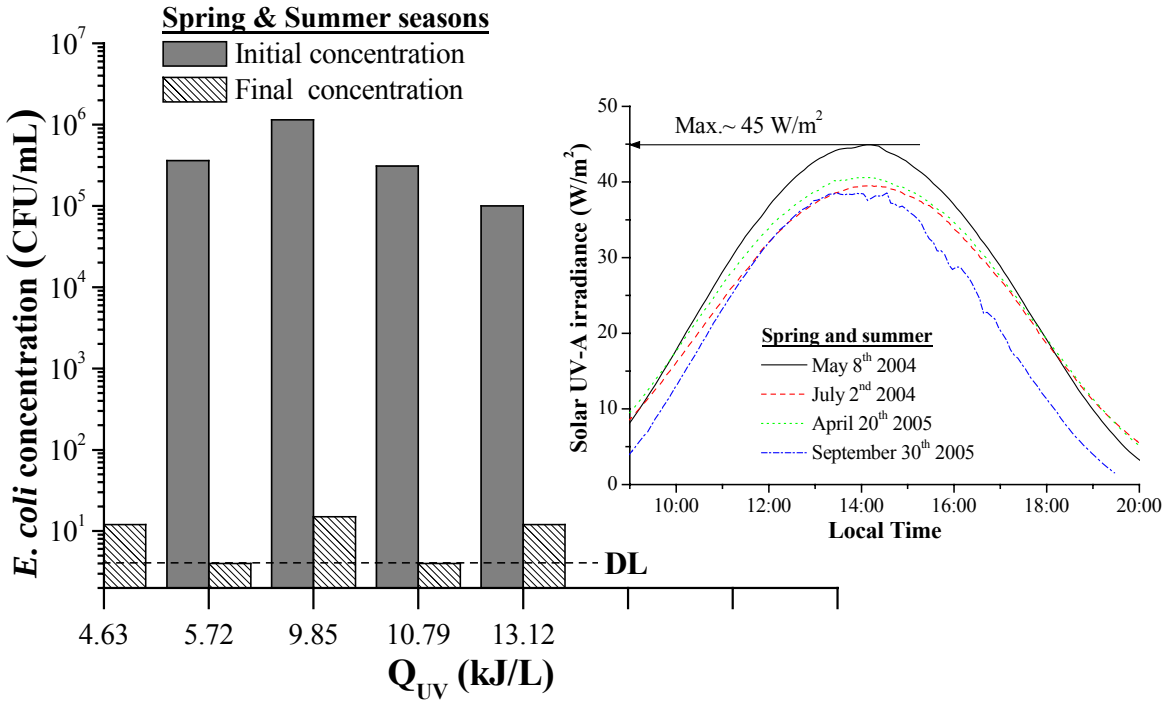

Figure 4.2 Inactivation of *E. coli* by sunlight over Q_{UV} in the 0.4-m^2 CPC reactor with the immobilized Ahlstrom catalyst during different experiments in spring and summer. Inset: solar UV irradiance over time on 4 days in spring and summer, 2004-2005 at the PSA. Each bar represents the average of the replicates.

Figure 4.3 shows bacteria disinfection in the seasons with the least solar irradiation, from 1st October to 1st March, 2004 and 2005, with Q_{UV} only between 3 and 4 kJ L. Bacteria inactivation is from 3 to 5 logs. Therefore the average autumn-winter disinfection yield was slightly lower than in the spring and summer. **Figure 4.3** shows that higher disinfection yields are achieved with more accumulated energy in autumn and winter; while in spring and summer, disinfection results are equally good regardless of the total amount of energy received.

The temperature in the reactor was higher (25-30 ºC) in spring and summer than in autumn and winter (15-21 ºC), but disinfection performance cannot be attributed mainly to a thermal effect, since there is only synergy between solar radiation and temperature above 45 ºC [McGuigan *et al.,* 1998]. Nevertheless the photocatalytic disinfection rate for *E. coli* has been shown to increase when the temperature varies from 23 ºC to 45 ºC [Rincón and Pulgarin, 2003].

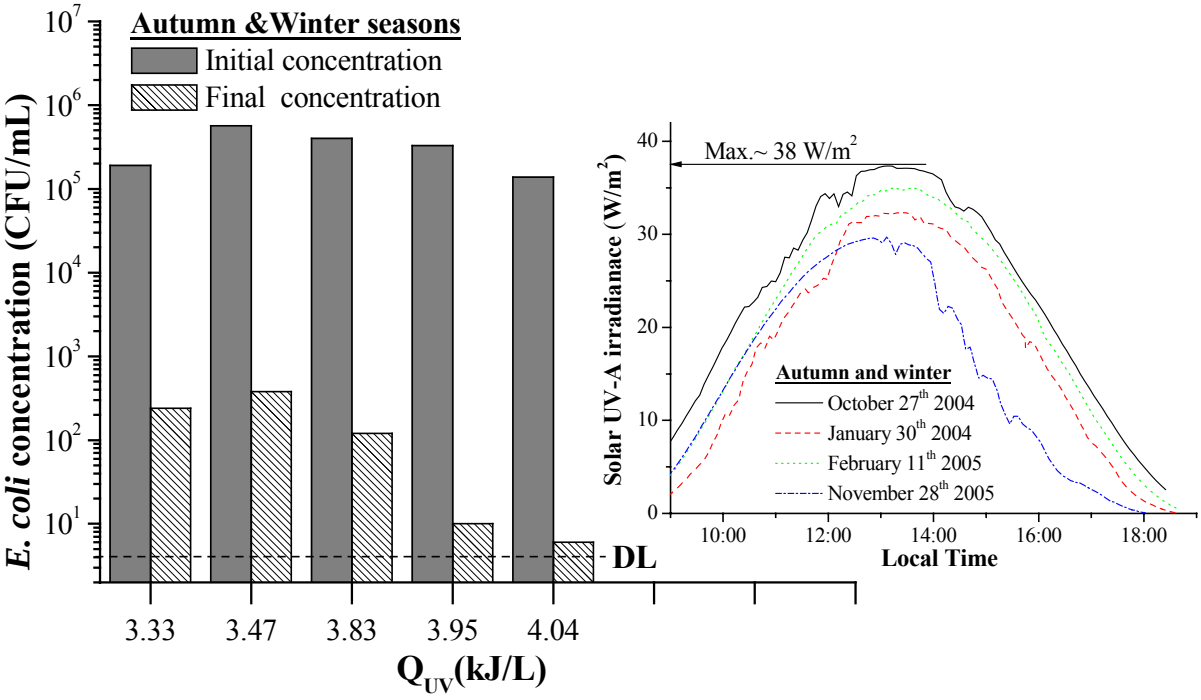

Figure 4.3 Inactivation of *E. coli* by sunlight over Q_{UV} in different experiments in the 0.4-m^2 CPC reactor with immobilized Ahlstrom catalyst in autumn and winter. Inset: solar UV irradiance over time on 4 days in autumn and winter of 2004-2005 at PSA. Each bar represents the average of replicates.

This experimental evidence points out once more that photocatalytic disinfection can produce certain disinfection with a minimum of UV photons. Below this there is a correlation between disinfection yield and UV energy received, and above, disinfection remains similar regardless of the energy received. Therefore, disinfection by photocatalysis is not exactly proportional to Q_{UV}. **Figures 4.2** and **4.3** show that 4 kJ/L seems to be the minimum Q_{UV} for 5-log inactivation of *E. coli* under these experimental conditions.

Other authors who have previously studied the dependency of photocatalytic disinfection on light irradiance at laboratory scale have generally found that up to a certain radiant flux, faster inactivation of *E. coli* occurs at higher light intensities (irradiance W/m^2) with square-root dependence of the disinfection rate on light intensity [Lee *et al.,* 1997; Bekbölet, 1997; Cho *et al.,* 2004].

In our winter experiments, in addition to the lower accumulated energy over the experimental time (J/L), lower average solar irradiance (W/m^2) also has to be taken into account. In this regard, the disinfection experiments shown in **Figure 4.3** still show proportional dependence of disinfection performance on overall irradiance, whereas in **Figure 4.2** irradiance can no longer be seen as the driving factor in the process. Similar slightly better disinfection in summer than in autumn was also reported by Rincón and Pulgarin, who also compared *E. coli* disinfection experiments in solar CPC reactors in different seasons and, like ours, found no linear correlation between disinfection yield and energy received at any other time of the year [Rincón and Pulgarin, 2004 a].

Effect of time of day on Fusarium inactivation: **Figure 4.4** shows solar-only and solar photocatalytic inactivation of *F. solani* under two different solar irradiance conditions. Both organisms have been demonstrated to be susceptible to solar-only and solar photocatlytic disinfection under natural and simulated sunlight [Lonnen *et al.*, 2005; Sichel *et al.*, 2007 a]. The experiments described in this article started at different times on the same sunny day and, therefore, with different solar irradiance. One test series (solar-only and photocatalysis) started at 08:30 local time, and the other series began at 10:15 local time. While previous results (**Figures 4.1-4.3**) had shown a 5-log reduction in bacteria after 1.5 h of treatment using immobilized catalysts, **Figure 4.4** shows that the

photocatalytic disinfection of *F. solani* using a TiO_2 slurry requires at least 3 h of solar exposure to reduce approx. 1300 CFU/mL down to the detection limit. Similar disinfection kinetics and total disinfection times for experiments performed under high solar irradiance have previously been reported for both *Fusarium* species, as well as *F. equiseti, F. verticillioides*, and *F. oxysporum* [Sichel *et al.*, 2007 b].

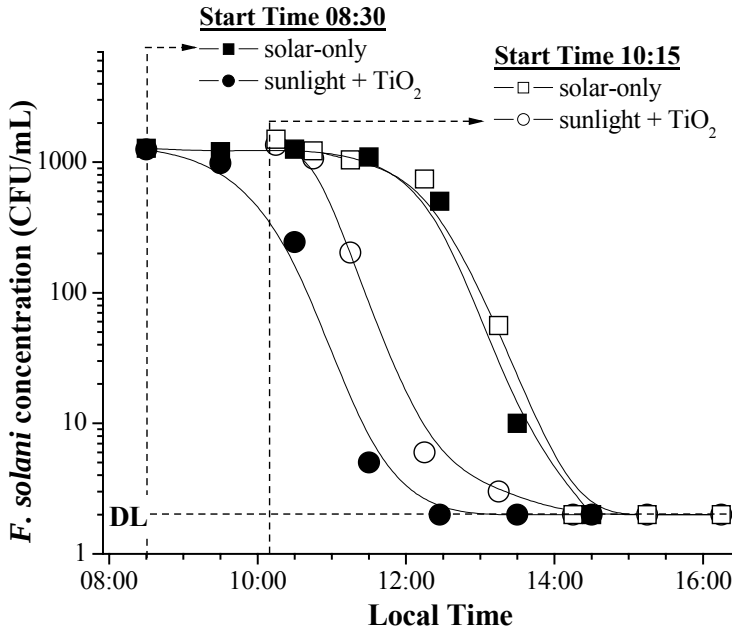

Figure 4.4 Photocatalytic inactivation of *F. solani* under natural solar radiation during experiments starting at 08:30 with (–●–) and without TiO_2 (–■–) and at 10:15 with (–○–) and without TiO_2 (–□–). Initial concentration 1.3 x 10^3 CFU/mL. Each point represents the average of triplicates.

A look at **Figure 4.4** shows much better performance by TiO_2 photocatalytic disinfection than solar-only. Inactivation of this fungus by photocatalysis is similar in both runs, but displaced in time, while the solar-only disinfection experiment has a two-hour lag in the first experiment. **Figures 4.5 a** and **4.5 b** show the hourly average UV irradiance (solid bars) and the solar UV dose (bars with diagonal fill) during the series shown in **Figure 4.4**. The experiment that started at 10:15 needed 485 kJ /m^2 for photocatalytic inactivation of 1300 CFU/mL down to the detection limit (2 CFU/mL), while the experiment that started at 08:30, which received a lower solar UV dose (259 kJ /m^2) because the average UV irradiance was lower, arrived at the same result after 4 hours of solar photocatalytic treatment.

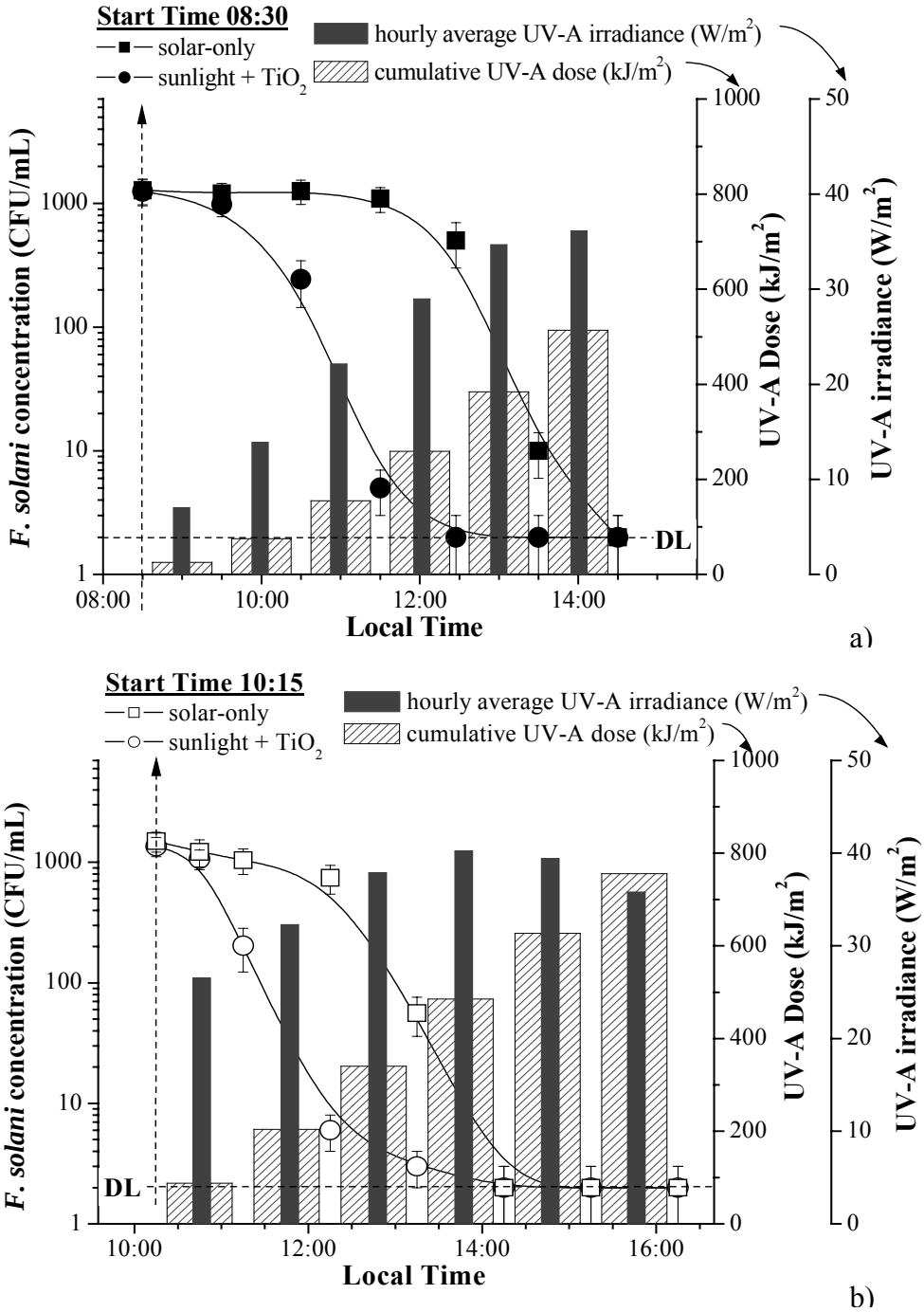

Figure 4.5 Effect of the cumulative solar UV dose (bars with diagonal fill) and hourly average solar UV irradiance (solid bars) on the photocatalytic inactivation of *F. solani* under natural solar radiation with (–●–, –○–) and without TiO_2 (–■–, –□–). Experiments started at 08:30 (a) and at 10:15 (b). Initial concentration of *F. solani* 1.3 x 10^3 CFU/mL. Each point represents the average of triplicates, and vertical lines show the statistical error at 95% confidence level.

The first points of the photocatalytic kinetics in **Figures 4.5 a** and **4.5 b** show the disinfection rate slightly more accelerated with high UV irradiance (**Figure 4.5 b**) than low UV irradiance (**Figure 4.5 a**). Nevertheless, once the system had received a certain UV dose, disinfection was similar in both cases, regardless of the total UV-dose received. Thus, a minimum UV dose or, with respect to future applications for solar reactor disinfection, a similar minimum Q_{UV} for disinfection can also be assumed for the photocatalytic disinfection of *F. solani*.

Solar-only disinfection is affected very differently by UV irradiance and dose. **Figure 4.5 a** shows that for 3 hours, when the average UV irradiance was below 25 W/m and the UV dose was 154 kJ /m^2 the *F. solani* concentration remained constant. When the UV irradiance rose to 32 W/m^2 and UV dose to 204 kJ/m (**Figure 4.5 b**), the concentration started to diminish after 2 hours of solar exposure. At least 6 hours of solar exposure were necessary to reach total abatement of the initial fungus concentration with a low irradiance yielding a solar UV dose of 514 kJ /m^2 (**Figure 4.5 a**). Whereas the inactivation with high UV irradiance took only 4 hours of exposure, reaching a similar solar UV dose of 485 kJ /m^2 (**Figure 4.5 b**). Therefore this result can be interpreted in two ways, a minimum UV irradiance (solar UV intensity) is required for solar-only disinfection, which, in the case under study, is around 30 W /m^2, or, that a certain solar UV dose is required, which is approx. 500 kJ /m^2, the corresponding Q_{UV} for solar reactors would be around of 8.25 kJ/L.

Fusarium inactivation under different weather conditions (sunny and cloudy): Another type of *Fusarium*, *F. anthophilum,* was employed in this study. This fungus was less resistant to photocatalytic treatment than *F. solani*. **Figure 4.6** shows two experiments, one performed on a completely sunny day (a) and the second on a cloudy day (b), with lower solar irradiance. The solar irradiance on the sunny day went from 20 to 43 W/m^2, while during the cloudy day it went only from 14 to 26 W/m^2. Photocatalytic disinfection on the sunny day took only 1 hour to reduce the fungus from 10^3 CFU/mL to the detection limit, but 2 h under cloudy conditions. A solar UV dose of 145 kJ /m^2, was enough to inactivate all fungus colonies on the cloudy day. Under sunny conditions, the UV-dose received after 1 hour was higher, 176 kJ/m.

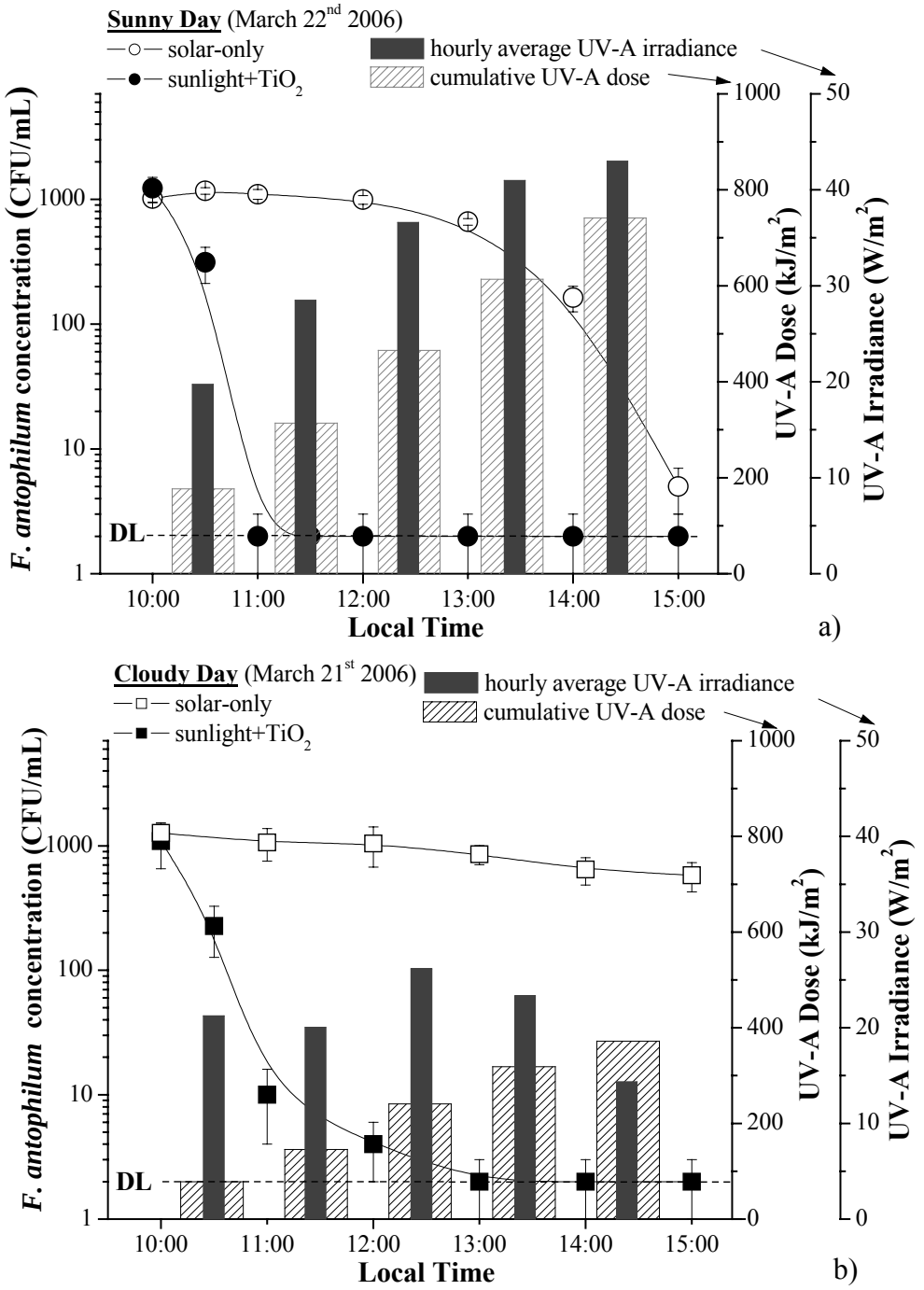

Figure 4.6 Effect of the cumulative solar UV dose (bars with diagonal fill) and hourly average solar UV irradiance (solid bars) on the photocatalytic inactivation of *F. anthophilum* under natural solar radiation with (–■–, –●–) and without TiO_2 (–○–, –□–). Experiments under sunny (a) and cloudy conditions (b). Initial concentration of *F. anthophilum* 1.0-1.3 x 10^3 CFU/mL. Each point represents the average of triplicates, and vertical lines show the statistical error at 95% confidence level.

The results of photocatalysis in **Figure 4.6** show similar disinfection kinetics under both sunny and cloudy conditions. On the other hand, the solar-only disinfection under both conditions (low and high solar irradiance) is clearly different. After 5 hours of solar exposure, the concentration of *F. anthophilum* is reduced from 1300 CFU/mL down to 600 CFU/mL under cloudy conditions, and from 1000 CFU/mL down to 5 CFU/mL on the sunny day. The UV dose was 372 kJ /m^2 on the cloudy day, and 741 kJ /m^2, on the sunny day. It can therefore be said that the UV dose necessary to disinfect this fungus is very low when TiO_2 is used regardless of the solar irradiation conditions, while solar-only disinfection requires a higher UV dose and therefore the difference in results under sunny and cloudy conditions is also greater. Furthermore, it may be observed that the necessary UV dose or intensity (irradiance) needed for disinfection of this fungus was only received on the sunny day. This result also underlines the importance of using TiO_2 to ensure the solar disinfection treatment of fungal spores under suboptimal conditions.

Conclusions

- Increasing the reactor surface does not necessarily lead to better disinfection performance. Once a solar photocatalytic system has received the necessary amount of UV energy, disinfection is reached regardless of the irradiated surface.

- Comparison of experiments in different seasons, early and later in the day, and under cloudy and sunny conditions, leads us to conclude that solar photocatalytic disinfection of *E. coli, F. solani,* and *F. anthophilum* does not depend proportionally on solar UV irradiance (solar UV intensity) as long as enough photons have been received for disinfection. The minimum UV energy necessary to reach a certain disinfection depends on the microorganism and the reactor configuration.

- Solar-only disinfection was found to be more dependent on irradiation conditions than photocatalytic disinfection. It requires higher minimum solar UV irradiance (solar UV intensity) and higher minimum UV dose for disinfection than solar photocatalytic disinfection.

Acknowledgements

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IV.5. Lethal synergy of solar UV-radiation and H_2O_2 on wild *Fusarium solani* spores in distilled and natural well water

Abstract

Environmentally-friendly disinfection methods are needed in many industrial applications. As a natural metabolite of many organisms, hydrogen peroxide (H_2O_2)-based disinfection may be such a method as long as H_2O_2 is used in nontoxic concentrations. Nevertheless, when applied alone as a disinfectant, H_2O_2 concentrations need to be high enough to achieve significant pathogen reduction, and this may lead to phytotoxicity. This paper shows how H_2O_2 disinfection concentrations could be reduced by using the synergic lethality, of H_2O_2 and sunlight. Experiments were performed on spores of *F. solani*, the ubiquitous, pytho- and human pathogenic fungus. Laboratory (250-mL bottles) and pilot plant solar reactors (2 x 14 L Compound Parabolic Collectors) were employed with distilled water and real well water under natural sunlight. The combination of H_2O_2 with solar UV-irradiation allowed H_2O_2 to be reduced from 500 mg/L to 5 mg/L with better disinfection results than in dark disinfection tests. Solar irradiation alone reduced fungal Colony Forming Units (CFUs) only slightly due to the low solar irradiance while the experiments were being performed. Combined solar and H_2O_2 disinfection was effective at laboratory and CPC reactor scale, with distilled and real water. In the past, the lethal synergy of H_2O_2 and UV-A light has only been reported for bacteria and virus *in vitro*. Sensitivity of *F. solani* spores is the first proof of the suitability of this method for the disinfection of members of the fungus kingdom. This opens the way to applications for agricultural water resources, seed disinfection, curing of fungal skin infections, etc.

Keywords: *F. solani*; solar radiation; hydrogen peroxide; solar reactors; natural well water.

Introduction

Modern life is affected by fungal pathogens in a variety of ways. In medicine, fungal pathogens, for example, *Fusarium* spp., cause diseases, e.g., skin diseases, especially in

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immunodeficient patients [Gupta *et al.,* 2000] , or eye infections due to fungal contamination of contact lenses [Zhang *et al.,* 2007]. Agriculture is probably the most affected field, as fungal pathogens, e.g., *Fusarium* spp., cause a large part of plant diseases and significant crop loss. Common vehicles for pathogens in greenhouses are irrigation water and crop seeds. In soilless cultures, where crops are planted on inert substances like rock wool [Bussell, 2004], and are fed by nutrient solutions, it is especially easy for pathogens to spread [Gómez, 2003]. One of the most important pathogenic *Fusarium* species in plants and humans is *F. solani*. It has been reported not only in soils throughout the world [Summerell *et al.,*1993; Sangalang *et al.,*1995] , but even in hospital water distribution systems [Anaissie *et al.,* 2001].

A variety of substances have traditionally been used to reduce the number of fungal pathogens, many of them non-biodegradable pesticides, which accumulate in soils and plants and later affect human health. Chlorine is still used as a universal disinfectant, but in addition to its phytotoxicity [Jarvis, 1998], organohalides in chlorinated drinking water attracted criticism for its use in the 1970s [Bellar *et al.,*1974]. Hydrogen Peroxide is also commonly used as a disinfectant in agriculture [Chikthimmah *et al.,* 2005], but in hydroponic cultures, is already phytotoxic at 50 mg/L [Coosemans, 1995]. Thus to improve its efficacy, H_2O_2 has been combined with germicidal UV-C radiation or ozone [Sharpless *et al.,* 2003]. Both techniques are in use, but the energy required is costly. Solar radiation can also be used for disinfection. Inactivation of many waterborne microorganisms, including bacteria [Acra *et al.,*1984], cysts [Heaselgrave *et al.,* 2006] and fungi [Lonnen *et al.,* 2005; Sichel *et al.,* 2007 b] has already been reported. Water is already treated in many sunny areas by solar disinfection, or SODIS.

The term SODIS particularly refers to the solar disinfection of drinking water in PET bottles on a small household scale [Wegelin *et al.,* 1994; Conroy *et al.,* 1996]. Disinfection of larger volumes is also possible in solar reactors [Sichel *et al.,* 2007 a]. The germicidal action of medium-UV and near-UV sunlight, reaching the earth, UV-B (290-320 nm) and UV-A (320-380 nm), has been studied for many years.

Near UV-light damages the DNA, mainly indirectly by absorption by intracellular chromophores, as already well documented [Favre, 1977; Moan and Peak, 1989; Wondrak *et al.,* 2006], and the resulting synthesising of Reactive Oxygen Species (ROS) [Cunningham *et al.,*1985; Vile and Tyrrell, 1995 and 1993]. ROS, mainly the superoxide anion and H_2O_2, induce oxidative stress in the cells through the Fenton and Haber-Weiss reactions, leading to oxidative DNA damage [Imlay *et al.,*1988; Imlay and Linn, 1988; Keyer, 1996; Filho and Meneghini, 1984] pyrimidine dimer formation [Tyrrell, 1973] and lipid peroxidation [Vile and Tyrrell, 1995]. The synergy of near UV-light and H_2O_2 leading to improved killing was first reported by Anathaswamy *et al.* [Anathaswamy *et al.,* 1979; Anathaswamy and Eisenstark, 1977], for phage T7. A following publication in 1980 reported the combined lethality of H_2O_2 and near UV- radiation on *E. coli* K12 [Hartman, 1980]. To date, there has been no work published demonstrating the existence of such lethal synergy on fungi.

This paper evaluates use of H_2O_2 (from 500 to 5 mg/L) in the presence of solar UV radiation for inactivation of *F. solani* spores. The experiments were carried out with two types of solar reactor: i) 250-mL glass bottles under natural sunlight, and ii) 14-L reactors using solar CPC mirrors to collect the solar radiation. Suspensions of *F. solani* in distilled and real well water were employed for the disinfection studies.

Materials and Methods

Fungal strain and quantification: A wild strain of *F. solani* was acquired from soil cultures in Almería (southern Spain). Fungal colonies were transferred to agar sporulation media containing potassium chloride, and kept at 25 ºC for 15 days to produce microconidia, macroconidia and chlamydospores. The same strain and sporulation method had been used previously [Sichel *et al.,* 2007 b]. The spores were washed from the mycelia and agar with sterile water. The resulting suspension was centrifuged at 2000 g for 10 min and washed three times with sterilized, distilled water. The spore concentration was determined with a counting chamber (Neubauer improved) using a phase contrast microscope. The spore suspension concentration in the reactor was adjusted to between 0.3-1 x 10^3 CFU/mL. Some 14-L CPC reactor experiments were performed with fewer spores than in the bottle reactor experiments, as the sporulation procedure used only permits a limited number of

CFUs. For sampling, 1-mL samples were taken from the reactor water and mixed with 100 µL of catalase at 0.1 g/L (Sigma Aldrich, USA) to avoid post irradiation inactivation by the H_2O_2 oxidant. The reduction in fungal concentration was measured as the decrease in cultivable fungal CFUs. 50-250 µL of the samples were plated out on acidified malt agar (Sigma Aldrich, USA). Where fewer than 10 colonies per plate were observed, 500 µL of the sample were plated to a detection limit of 2 CFU/mL. Each measurement was made in triplicate, with three sample inoculations on three different plates. The fungal colonies were counted after 2 days of incubation at 28 ºC in the dark. Measurement data were analysed by one-way ANOVA ($P<0.05$, Confidence > 95%, Origin v7.0300, OriginLab Corp., Northampton, USA).

Solar compound parabolic collectors: All experiments were performed under natural solar radiation at the Plataforma Solar de Almería, Spain, located at 37º84' N and 2º34' W. The solar CPC reactors were designed and built by AO SOL (Lisbon, Portugal) [Collares-Pereira, 2006]. The system (**Figure 5.1**) consists of two borosilicate glass tubes in the focus of the CPC reflectors. Glass tubes and CPC collectors are held by aluminum frames mounted on platforms tilted at 37° local latitude. The glass tubes are connected in series so that water flows directly from one to another and finally into a tank. A centrifugal pump (20 Watts, Panworld, Spain) then returns the water to the solar collector. The tank has an opening on top where contaminated water is poured in.

For the disinfection process, this opening is closed with a plastic lid. The treated water is later recovered by opening the outlet valve. The photoreactor volume is 14 L, the illuminated volume 4.7 L, and the irradiated collector surface 0.42 m^2. CPC reactors are often used for solar photocatalytic water treatment due to their high capacity for providing solar UV-irradiation homogenously during reactions [Malato *et al.,* 2002 a, b].

The fungal suspension was left recirculating along with the H_2O_2 for 15 min in the dark reactor (adaptation and homogenization time). Then the first sample was taken and the reactor was exposed to solar irradiation. For the CPC experiments, all samples were first taken in 50-mL tubes and then mixed with catalase in 1.5-mL Eppendorf tubes. The first 50-mL sample was kept in the dark at room temperature and analysed again at the end of

the experiment to determine CFU and H_2O_2 losses in the dark. The water temperature in the reactor was monitored, but not controlled, during all the experiments. All H_2O_2 experiments were performed simultaneously with solar-only disinfection (blank) tests using twin solar CPC reactors installed at the Plataforma Solar de Almería for comparison under the same conditions. The experiments were repeated three times. Disinfection conditions can vary significantly on different days due to changes in irradiation, fungus spores and temperatures. For this reason, the reactor experiments are not presented as an average of the three repetitions but as one representative experiment of the three repetitions with the sample errors and against its simultaneous blank experiment.

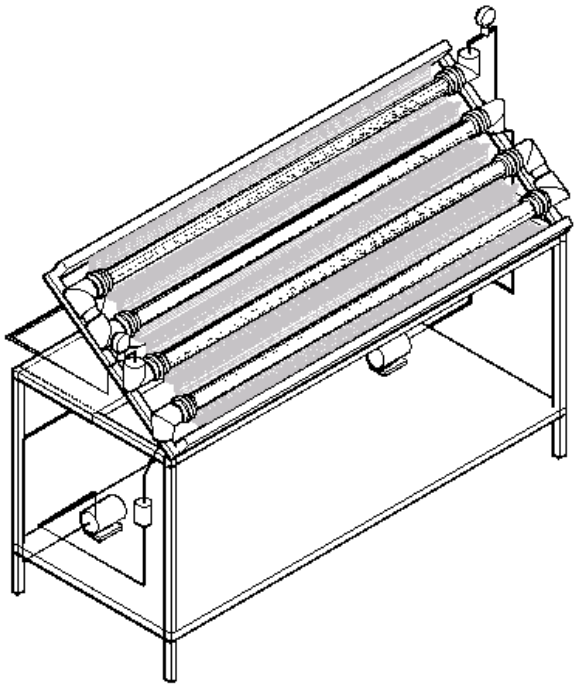

Figure 5.1 Drawing of CPC reactor with tubes, and two pumps for water recirculation in the two equal experimental circuits.

Solar Bottle reactors: The solar bottle reactors were 250-mL DURAN-Glass bottles (Schott, Germany) exposed to natural sunlight. DURAN-Glass covers (Schott) were used instead of plastic lids, to allow the solar radiation to enter the bottle reactor from all directions. The bottles were stirred during the experiment with magnetic stirrers at 100 rpm on a horizontal platform. Solar photocatalytic and solar-only disinfection tests were carried out simultaneously in triplicate. The solar experiment control bottles (one with and another without H_2O_2) were stored at similar temperatures, but in the dark, to evaluate possible

dark inactivation due to temperature or H_2O_2 exposure. After 15 min adaptation time in the presence of H_2O_2, the bottle reactors were exposed to solar irradiation. UV-irradiance was measured with a global UV radiometer (Model CUV3, KIPP&ZONEN, Netherlands, with a typical sensitivity of 264 μV W^{-1} /m^2), on a horizontal platform. The average irradiance is given in W/m^2.

H_2O_2 measurement: H_2O_2 (Riedel-de Haën, Germany) at 35%wt. was used as received and diluted directly into the reactor water. Its concentration was measured in a spectrophotometer (PG Instruments Ltd T-60-U) at 410 nm with the help of the yellow complex formed with Titanium (IV) oxysulphate and H_2O_2 in glass cuvettes with a 1 cm path length. The Titanium (IV) oxysulphate method is used for drinking water analysis and has a 0.1 mg/L detection limit (DEV). The signal was read after 5 min incubation time against an H_2O_2 standard curve measured at the same time along with samples and was linear in the 0–100 mg/L concentration range. Titanium (IV) oxysulphate solution (Riedel-de Haën, Germany) was used as received. H_2O_2 concentrations were measured in the CPC reactor experiments, in which enough sample volume was available for analysis.

Well water experiments: These studies were carried out using natural well water so that the chemical environment in which inactivation took place would be as much like the irrigation water where phytopathogenic fungus may appear as possible. The water was taken from an approx. 200-m-deep well located near the PSA. Distilled water was used as a reference. **Table 5.1** shows the general physicochemical well-water parameters averaged over the period of the experimental series. This water can be distinguished from other water sources by its high carbonate content. The presence of other fungal species was not detected after applying the same malt agar detection method as for the experiments. Anion analysis was done by ion chromatography (IC) with a Dionex DX-600 system, and for cations with a Dionex DX-120 system. TOC and TC were analysed using a Shimadzu TOC-5050 a. The pH was measured by a pH electrode (WTW, Germany). Turbidity was measured with a laboratory turbidimeter Model 2100N (Hach, USA).

Table 5.1 Chemical characteristics of the PSA- well water. The values correspond to the average of the samples taken during the experimental series.

Parameter	Unit	Value
Sulfate	$mg_{SO_4^{2-}}$ /L	270
Nitrate	$mg_{NO_3^-}$ /L	12
Chloride	mg_{Cl^-} /L	300
Orthophosphates	$mg_{PO_4^{3-}}$ /L	37
Hydrogen carbonates	$mg_{CO_3^-}$ /L	650
Sodium	mg_{Na^+} /L	545
Potassium	mg_{K^+} /L	18.5
Total Organic Carbon [TOC]	$mg_{C_{org}}$ /L	11
Turbidity	NTU	5
pH	-	7.8

Results

To measure the reduction in fungal CFUs in darkness, *F. solani* spores were exposed to hydrogen peroxide at concentrations of 0, 5, 50, 100 and 500 mg/L H_2O_2 (**Figure 5.2**). The spores were stirred in 250 mL glass bottle reactors for 5 h. Samples were taken after 15 min (adaptation time), after 3 h and 5 h. The temperature was kept at 25 °C ambient temperature. In the blank experiment, containing only *F. solani* spores and distilled water, the concentration of fungal CFUs remained stable. For hydrogen peroxide exposure, dark inactivation of fungal suspensions increased with increasing peroxide concentration and experimental time. At hydrogen peroxide concentrations of 5 mg/L and 50 mg/L, the *F. solani* spore concentration was still unaffected after three hours. After five hours, H_2O_2 concentrations of 5 mg/L were able to reduce fungal CFUs slightly (0.2 log). The 50 mg/L H_2O_2 concentration produced a 0.4-log reduction after 5 h.

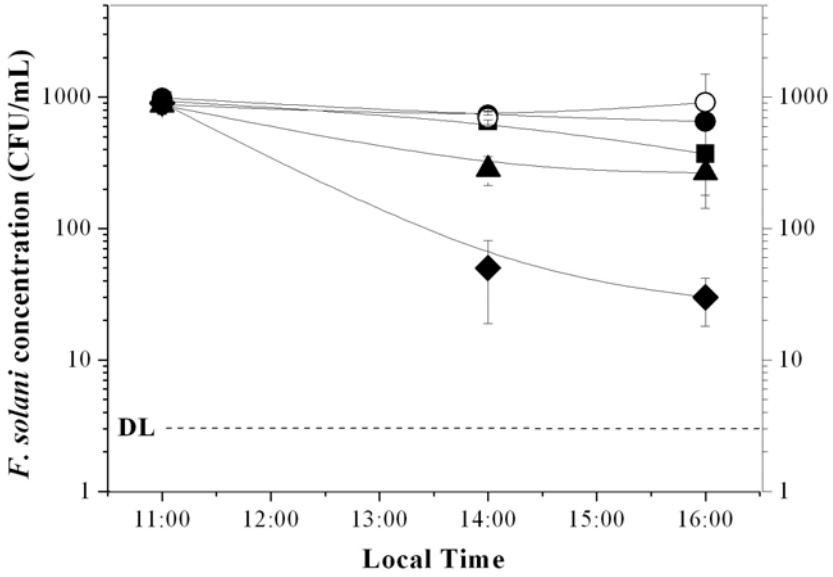

Figure 5.2 Exposure of *F. solani* to H_2O_2 concentrations from 5-500 mg/L with distilled water in darkness and in bottle reactor. H_2O_2 concentrations: 0 mg/L (–○–), 5 mg/L (–●–), 50 mg/L (–■–), 100 mg/L (–▲–), 500 mg/L (–♦–). Each point represents the average value of triplicate experiments; the bars show the statistical error that yields a 95 % confidence level.

Exposure to 100 mg/L H_2O_2 already showed a slight effect on spore concentration, with a 0.5 log reduction after 3 h and 0.6 log reduction by the end of the experiment. *F. solani* suspensions were reduced 1.3 logs by 500 mg/L H_2O_2 after 3 h and 1.5 logs by the end of the experiment. In no experiment was the detection limit of 2 CFU, corresponding to a 2.7 log reduction in fungal CFUs, reached.

Exposure of *F. solani* spores to solar irradiation at the same H_2O_2 concentrations as described above enhanced killing at 5 mg/L H_2O_2. **Figure 5.3** shows the decrease in fungal CFUs with increasing H_2O_2 concentration from 0, 5, 50, and 100 up to 500 mg/L. The temperature was 28-33.4 °C and did not affect the dark control. The average UV-irradiance was 30.4 W/m^2 and solar inactivation was only able to reduce the fungal concentration of the blank test (no H_2O_2 added) by 0.7 log. In the experiments with added H_2O_2, the fungal CFUs were reduced to the detection limit (2.7 logs) at all H_2O_2 concentrations tested. Enhanced killing of fungal spores observed in the solar disinfection with H_2O_2 can be interpreted as a synergic killing effect. This means that the total reduction in CFU was greater than just added H_2O_2 in the dark (0.2 log at 5 mg/L of H_2O_2)

and solar inactivation (0.7 logs) together, but a strongly enhanced synergy of both, accomplishing complete inactivation.

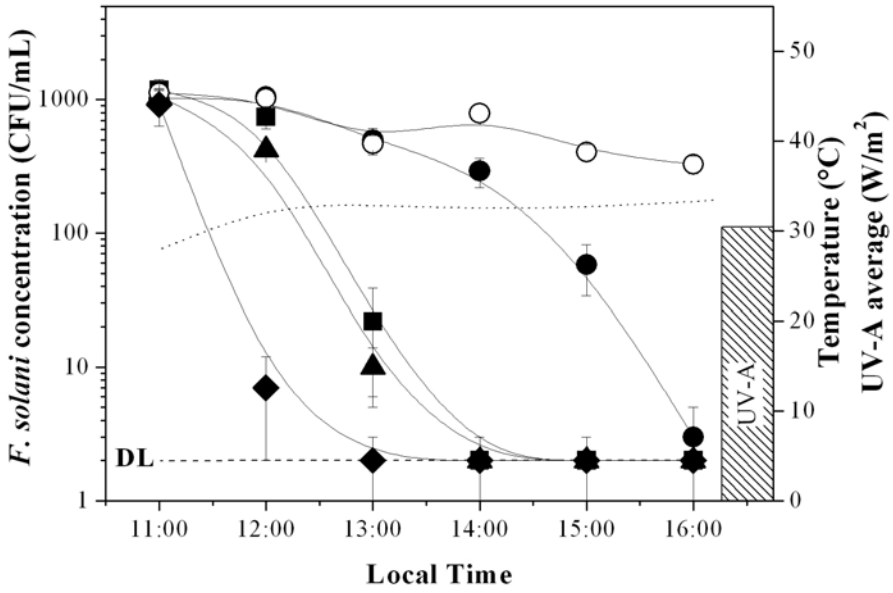

Figure 5.3 Exposure of *F. solani* to H_2O_2 concentrations from 5-500 mg/L and natural sunlight with distilled water in bottle reactors. The H_2O_2 concentrations were 0 mg/L (–○–), 5 mg/L (–●–), 50 mg/L (–■–), 100 mg/L (–▲–), 500 mg/L (–♦–). Each point represents the average value of triplicate experiments; the bars show the statistical error that yields a 95 % confidence level. Dashed line represents temperature and column average irradiance during the experiments.

While the fungal concentrations in the presence of H_2O_2 and sunlight always reached the detection limit, the shoulder of the disinfection curve got smaller with increasing H_2O_2 concentrations. This shoulder can be attributed to the resistance of the fungus spores to oxidative stress. The higher the H_2O_2 concentration, the faster fungal resistance was overcome. This led to a reduction of the total time needed for complete fungal disinfection from 5 h for the 5 mg/L H_2O_2 concentration to 2 h for the 500 mg/L concentration. The reason that lag phases decrease with higher H_2O_2 concentrations might be the faster diffusion-mediated influx of H_2O_2. In fact, H_2O_2 penetration of bio- membranes is a recent vividly discussed topic (see Discussion).

Upscaling from the 250-mL test bottles to larger volumes like our 14-L CPC reactors always implies more difficult disinfection. Exposing more water limits penetration of sunlight during treatment due to scattering and absorption, depending on the turbidity of

the water. Because of this, larger volumes are recirculated to receive sunlight in a solar reactor module, such as our CPC reactors.

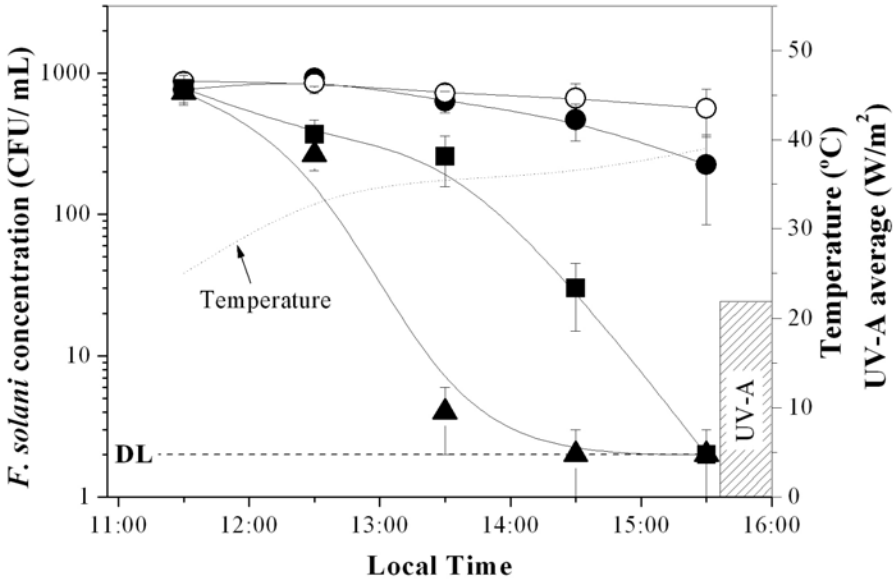

Figure 5.4 Exposure of *F. solani* to H_2O_2 concentrations of 50 mg/L (–■–) and sunlight in the solar CPC reactor with distilled water compared to blank test without H_2O_2 (–○–). Dark control samples with (-+-) and without (-x-) H_2O_2. Each point represents the average value of samples taken on one representative reactor experiment; the bars show the statistical error that yields a 95 % confidence level. Dotted line represents temperature, dashed line H_2O_2 concentration in the light experiment and solid line the H_2O_2 concentration of the dark control. The column is the average irradiance during the experiments.

As the water is recirculated through the solar collector, light penetrates it completely, but when the spores in the water go through the tank and tubing, they are protected from the detrimental solar irradiation. As microorganisms that only receive sublethal UV-A irradiation become more resistant to the UV-A-induced oxidative stress [Hoerter *et al.*, 2005], microorganisms may have a chance to partly recover and adapt more easily to the induced oxidative stress. This explains why longer treatment times and higher H_2O_2 concentrations were needed for CPC reactor disinfection. **Figure 5.4** shows a CPC disinfection experiment upscaled to 14 L of distilled water.

The addition of 50 mg/L H_2O_2 reduced the *Fusarium* CFUs by 2.6 logs. The blank experiment without H_2O_2 reduced the fungal spores 0.8 log with the same average irradiance of 23 W/m^2. The H_2O_2 concentration decreased almost linearly during the

experiment from 49.8 mg/L to 31.7 mg/L. In the dark experiments, the H_2O_2 concentration only decreased slightly (2.5 mg/L). Lyses of H_2O_2 by UV light can only be expected from UV-C wavelengths and is therefore not the reason for the reduction in H_2O_2 (since there is no UV-C radiation in the solar spectrum). The temperature, which was from 25 to 33 °C during solar exposure, has a completely different profile from H_2O_2 consumption and is also too low to be the reason for its disappearance. The reaction H_2O_2 consumption profile is similar to Fenton-like or even photo-Fenton-like decomposition of H_2O_2 in degradation of chemical compounds in CPC reactors [Malato *et al.,* 2002 a, b]. The influence of the Fenton reaction in the process will be considered further in the Discussion. Slight H_2O_2 losses (0.1 log) in the dark can be attributed to enzymatic decomposition by the fungal spores [Sousa lopez, 2004].

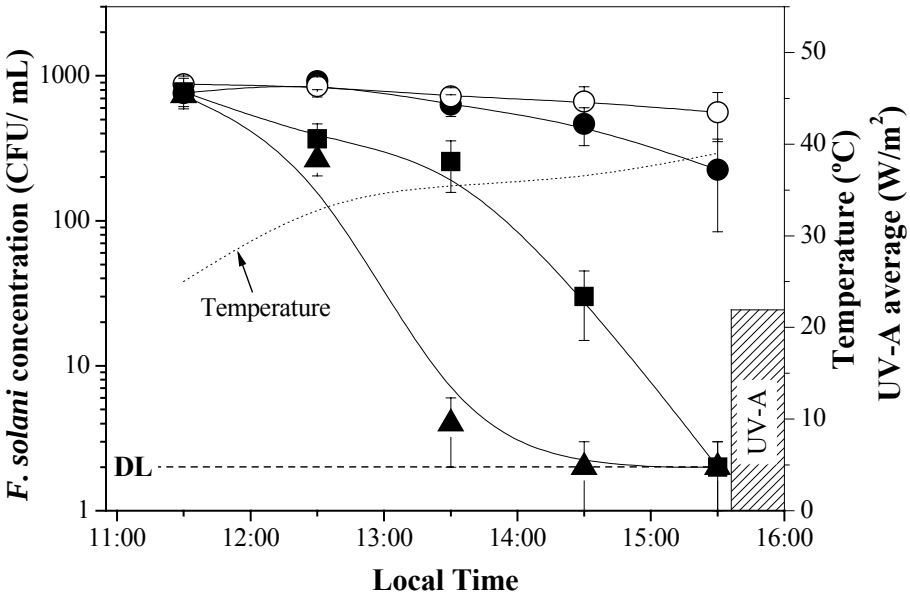

Figure 5.5 Exposure of *F. solani* to H_2O_2 concentrations of 5 mg/L (–●–), 10 mg/L (–■–), 50 mg/L (–▲–) under sunlight in bottle reactors with well water compared to blank test without H_2O_2 (–○–). Each point represents the average value of triplicate experiments; the bars show the statistical error that yields a 95 % confidence level. Dashed line represents temperature and column average irradiance during the experiments.

Disinfection reactions are always altered when real water is compared to distilled water. The impact on disinfection in the case of the H_2O_2 and solar treatment could be expected to be mainly due to the ion charges, pH and turbidity of the water. Experiments with local well water were the closest approach to solar H_2O_2-enhanced disinfection in local greenhouses. Therefore, water with the physicochemical characteristics in **Table 5.1** was

used in bottle reactor experiments. The bottle reactors in **Figure 5.5** were exposed to sunlight with H_2O_2 concentrations of 0, 5, 10 and 50 mg/L.

The temperature was from 25 to 39.3 °C, constantly increasing throughout the experiment. The control bottles stirred in darkness at similar temperatures did not show any *Fusarium* inactivation which excludes the possibility that fungal spore inactivation was due to heating. The average UV irradiance on this winter day was 22 W/m^2. The fungal concentration decreased in the blank experiment 0.4 log.

With the addition of 5 mg/L H_2O_2, fungal spore reduction was 0.8 log and with 10 mg/L H_2O_2 the spore concentration reached the detection limit (3 log-reduction). In this case, the blank experiment for solar-only disinfection was apparently less affected than the H_2O_2 and sunlight disinfection experiment, probably due to well water properties. In the distilled water experiment in **Figure 5.3,** with an average UV irradiance of 30.4 W/m^2, the reduction of the blank was 0.7 log compared to the 0.4 log reduction in well water with 22 W/m^2. The lower irradiance cannot be expected to affect fungal viability linearly [Sichel *et al.,* 2007 c] but provides a reasonable explanation for less inactivation in well water. In the hydrogen peroxide experiments at H_2O_2 concentrations of 5 mg/L, fungal CFU-reduction in well water was only 0.8 log, whereas in distilled water, the detection limit (2.7 log decrease) was already reached at that concentration. Perturbation of H_2O_2 action may be due to several different reasons. Competitive oxidation reactions could coexist in the well-water matrix.

Furthermore, osmotic stress on microorganisms in distilled water has been shown to strongly influence disinfection in CPC reactors, probably due to damage to the cell wall osmo-regulative system [Sichel *et al.,* 2007 a]. In the case of *F. solani*, permeability of the fungal-spore cell wall could increase in distilled water, leading to faster influx of H_2O_2 into the cells. This also explains why the inactivation curves found for well water are similar to distilled water, but require higher H_2O_2 concentrations.

The upscaled experiment with well water in CPC reactors was the closest to real disinfection conditions in local greenhouses. Both solar-only and solar H_2O_2 combined

disinfection were less effective under low irradiance. Nevertheless, in the 14-L CPC solar reactor experiment with natural well water, strongly enhanced disinfection was still achieved with the synergic solar H_2O_2 treatment (**Figure 5.6**).

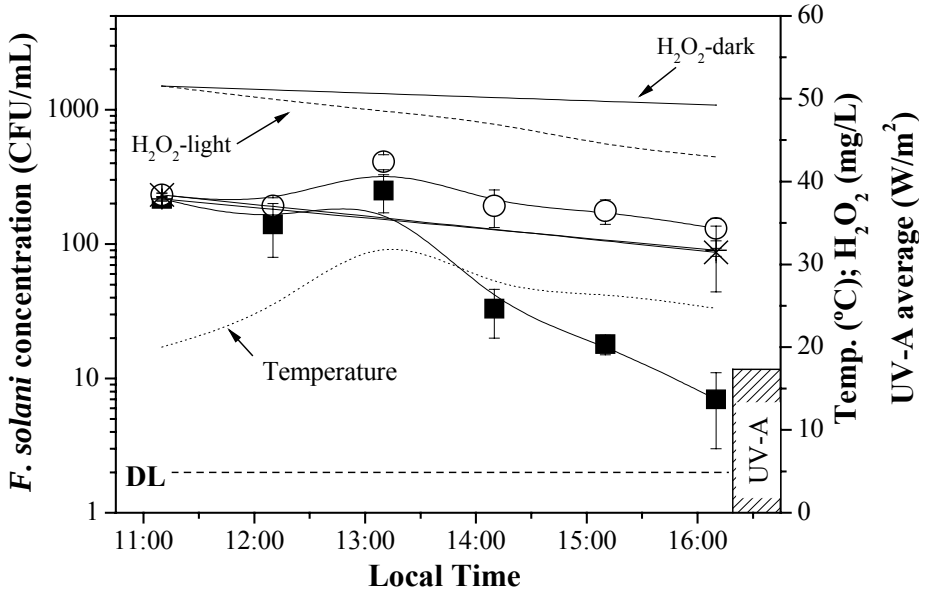

Figure 5.6 Exposure of *F. solani* to H_2O_2 concentrations of 50 mg/L (–■–) and solar radiation compared to blank test without H_2O_2 (–○–) with well water in the solar CPC reactor. Dark control samples were kept with (-+-) and without (-x-) H_2O_2. Each point represents the average value of samples taken on one representative reactor experiment; the bars show the statistical error that yields a 95 % confidence level. Dotted line represents temperature; dashed line H_2O_2 concentration during the solar experiment; solid line represents H_2O_2 concentrations of dark control. The column is the average irradiance during the experiments.

Solar irradiance of 17.1 W/m^2 reduced the fungal concentration in the blank solar disinfection experiment 0.4 log compared to a 0.8 log reduction in the distilled water CPC blank experiment (irr= 23 W/m^2). In the experiment with 50 mg/L H_2O_2, *Fusarium* concentrations were reduced 1.5 logs, from 0.3x10^3 CFU/mL to almost the detection limit, noticeably less than the total reduction in the distilled water experiment (2.6 logs). H_2O_2 consumption was also significantly reduced during the reaction from 51.5 mg/L to 43 mg/L. The decrease in H_2O_2 consumption indicates that Fenton decomposition of H_2O_2 might have been inhibited. The CPC experiments with interrupted low solar irradiance, was probably insufficient to induce the increase in intracellular free iron (see Discussion).

This means that there was no catalyst for H_2O_2 decomposition or hydroxyl radical production, and therefore, the synergistic inactivation of *F. solani* was strongly inhibited. Under such low irradiation, the turbidity of the well water would negatively affect the disinfection results, even though this was not clear in the strongly irradiated well-water bottle-reactor experiments. In addition, competitive well-water and H_2O_2 reactions, may also have contributed to limiting disinfection, even though the low dark H_2O_2 consumption (**Figure 5.5**) did not show such reactions.

Discussion

All living cells are exposed to intracellular H_2O_2 from their own metabolisms at steady-state concentrations, as determined by the rate of intracellular H_2O_2 production, its diffusion either into the cytosolic space and/or mitochondrial matrix; and thirdly, its effective removal by intracellular antioxidants [Cadenas and Davies, 2000]. The physiological concentration of H_2O_2 can vary depending on growth and oxidative stress conditions. In mammalian cells, it is usually assumed to be around 10^{-8} to 10^{-7} M [Chance *et al.,* 1979]. At steady-state concentrations of 1.0–3.0 μM H_2O_2 in the cytosol (approx. 7-21 μM extra-cellular H_2O_2), Jurkat T-cells develop programed cell death (apoptosis) probably triggered by radical production in Fenton-type reactions [Antunes and Cadenas, 2001].

Exogenous millimolar H_2O_2 concentrations kill logarithmically growing *E. coli* cells [Imlay *et al.,* 1986; Imlay and Linn 1988, Imlay *et al.,*1988]. Imlay *et al* (1986) distinguished between H_2O_2 induced Mode one (1 to 3 mM H_2O_2) and Mode two (over 20 mM) killing. Mode one killing was also attributed to DNA damage in Fenton-like reactions Mode one killing was also attributed to DNA damage via Fenton-like reactions [Imlay *et al.,* 1986; Imlay *et al.,* 1988].

In our results (**Figure 5.2**), the extra-cellular H_2O_2 concentrations inducing a slight decrease in concentration were between 0.15 to 1.5 mM with incubation of up to 5 h. Significant reductions occurred after 3 h at 3 mM and 14.7 mM. Why are the H_2O_2 concentrations for killing *F. solani* spores much higher than reported for exponential phase *E. coli* and Jurkat T-cells? In general, it is assumed that fungi have acquired a powerful

defence against oxidative stress [Li *et al.,* 2008; Medentsev *et al.,* 2001], also to protect themselves from the ROS produced by plants as a defensive response [Murphy *et al.,* 1998; Dangl *et al.,* 1996].

We think that on one hand, the strong resistance of *F. solani* spores might be attributed to an antioxidant response developed by them. But on the other hand, it might be due to the strong fungal cell wall. Protection by the cell wall probably induces a steep gradient between intracellular and extra-cellular H_2O_2. Recent publications report such gradients between outer and intracellular H_2O_2, or between different intracellular compartments for eukaryotic and prokaryotic cells [Seaver and Imlay, 2001; Antunes and Cadenas, 2001; Sousa lopez, 2004]. The concept that H_2O_2 freely diffuses across biomembranes found in the literature [Chance *et al.,* 1979] has therefore been abandoned. These H_2O_2 gradients can be positively correlated with cell survival for a variety of cells [Medensev *et al.,* 2001; Sousa lopez, 2004]. This also explains why stationary-phase cells, having a thicker and less permeable cell wall than exponential-phase cells, [De Nobel *et al.,* 1990; De Nobel and Barnett, 1991; Klis *et al.,* 2002] survive better under H_2O_2 exposure [Sousa lopez 2004].

For example, stationary phase *S. cerevisiae* cell permeability to H_2O_2 is reduced five times more than exponential phase cells [Sousa lopez 2004]. Therefore the thick-walled and very resistant *F. solani* spores, [Beyer *et al.,*2005] in our experiments are probably well protected from H_2O_2 influx. The small difference between inactivation after 3 and after 5 hours (**Figure 5.2**) might also be attributed to decreasing cell-wall permeability during the adaptation of the spores to exogenous H_2O_2. Similar responses have been reported for *S. cerevisia*e, in which cell-wall permeability, actively regulated by aquaporins [Bienert *et al.,* 2007] is thought to be part of an adaptive response to oxidative stress [Sousa lopez, 2004].

The enhanced killing of non-lethal concentrations of H_2O_2 together with near-UV light (**Figure 5.3-5.6**) was first reported in 1977 and 1979 [Anathaswamy *et al.,* 1977 and 1979] for phage T7. In later work, Hartman and Eisenstark [Hartman and Eisenstark, 1980] confirmed near-UV+ H_2O_2 (at 0.05%) sensitivity for stationary phase cells of *E. coli* K12. In these works, the synergic effect of near-UV+ H_2O_2 was thought to induce DNA-protein crosslinkage via alteration of –SH on cysteine, yielding a new chromophore with a λ_{max}

over 305 nm. This intracellular chromophore was suspected to increase synthesis of intracellular ROS (mainly superoxide anion and H_2O_2) [Mello Filho, 1984; Cunningham *et al.,* 1985] and therefore lethal damages to the cell as it is the case for other intracellular chromophores [Tyrrell, 1973; Vile and Tyrrell, 1995; Moan and Peak, 1989; Wondrak *et al.,* 2006].

While the superoxide radical has been found to be induced by intracellular UV-A absorption [Cunningham *et al.,* 1985] intracellular $^{\bullet}OH$ radical formation can be attributed to the univalent reduction of H_2O_2 postulated by Fenton and Haber-Weiss [Minotti and Aust, 1984; Mello Filho and Meneghini, 1987] in the presence of transition metals at acid pH, or Fenton-like reactions at neutral pH. The UV-A-induced release of labile iron in human skin fibroplasts [Pourzand *et al.,* 1999; Vile and Tyrrell, 1993] was reported in 1999, hypothesizing that iron was released from ferritin. How and why oxidative stress changes intracellular free iron levels in prokaryotic and eukaryotic cells and where the iron is released, has been an issue discussed in a large number of scientific publications to date [Vile *et al.,* 1995; Pourzand *et al.*, 1999; Cadet *et al.,* 2005; Andriopoulos *et al.,* 2007; Varghese *et al.,* 2007; Tyrrell and Reeve, 2006].

What is known is that the released "free iron" catalyses cell-damaging Fenton and Haber-Weiss reactions [Minotti and Aust, 1987; Mello Filho,1984; Keyer and Imlay 1996]. Recently, more and more publications, mostly about on *E. coli*, explain the increase in "free iron" in cells after oxidative stress (e.g. induced by UV-A challenge) by reactions between ROS and iron-sulphur clusters of iron-regulating proteins, like Fur or m-aconitase. ROS attacks on proteins sensitive to oxidative stress can directly liberate an iron atom from the cluster or indirectly increase the iron levels, causing changes in the transcriptional iron regulation [Varghese *et al.,* 2007; Beinert *et al.,* 1996; Kiley and Beinert, 2003].

The best-understood eukaryont most closely related to fungi, is the yeast *S. cerevisiae. S. cerevisiae* also shows predominantly transcriptional iron transport control [Eide *et al.,* 1992; Felice, 2005]. and its iron regulation system has also recently been shown to correspond to intact iron-sulphur clusters rather than to the real iron concentration [Chen *et al.,* 2004]. This means that *S. cerevisiae* probably also responds to ROS stress with iron up-

regulation and therefore increases damage through Fenton and Haber-Weiss in the presence of H_2O_2.

The lethal synergy of H_2O_2 and sunlight in our experiments with *F. solani* spores may therefore be attributed to the combined release of bound intracellular iron after oxidative stress in the presence of H_2O_2. H_2O_2 is decomposed by the Fenton reaction generating hydroxyl radicals, and leading to improved disinfection. This also explains the much greater decrease in H_2O_2 concentration in the solar reactor experiments compared to the dark control, as Fenton H_2O_2 decomposition only takes place in the presence of released iron, i.e., after oxidative stress.

The experiments (**Figures 5.4 and 5.6**) upscaled from 250-mL bottle reactors to the 14-L pilot plant showed that this method is suitable for water disinfection in irrigation systems or nutrient solutions in soilless cultures requiring many liters a day. Two solar CPC reactor modules were used with 14 L in each module. The volume of treated water and the number of CPC modules can be increased to meet larger disinfected water requirements. Several CPC systems can also be used simultaneously or connected in series. The use of two types of water (distilled and natural well water) in our study shows that synergic killing not only works under specially prepared conditions, but can also be applied to natural water. In previous work, solar reactor disinfection with 10 mg/L H_2O_2 in real water had been reported for *E. coli* [Rincón and Pulgarin, 2005]. Enhanced killing of *Fusarium* spores in our work is more noticeable than for *E. coli* because *E. coli* was also easily inactivated by low solar irradiation.

This contribution only demonstrates the disinfection of *F. solani* in water by H_2O_2 and sunlight, however, we think that it could also be a novel photo-promoted treatment against fungal skin infection. Photodynamic Treatment (PDT) is an emerging therapeutic procedure based on the use of photosensitizers, compounds that generate ROS on irradiation with visible light (usually photoactive dyes). PDT has been shown to be effective against skin cancer and fungal skin diseases [Calzavara-Pinton *et al.,* 2005].

The synergic effect of H_2O_2 and sunlight could inactivate fungal skin pathogens without risking degradation products from dyes. Secondly, as *F. solani* is a common pathogen causing eye diseases from the use of contaminated contact lenses [Zhang *et al.,* 2007], combined H_2O_2 and near UV light for lens disinfection is another possible application. And thirdly, improved seed disinfection in the presence of H_2O_2 and sunlight is possible, as fungi on the surface of seeds are often passed to the soil.

Acknowledgements

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V. Photocatalytic water disinfection with TiO_2 slurry for *Fusarium solani*: Upscale in solar CPC Reactors

This chapter presents the results of fungal spore inactivation with TiO_2 in 250mL-bottles and 14L CPC-reactors. Fungus inactivation in the CPC system does not reach the detection limit and therefore a new 60-L pilot plant was designed. These results have not yet been published, because it is expected that the results with the new pilot plant will provide more complete knowledge about the scaling-up process and better disinfection yields will probably be achieved in the improved system. Therefore, these results will be published when the new pilot plant has been evaluated and the experimental section of this work can be completed. The pilot plant is currently under construction and is expected to go into operation in October 2008. Nevertheless, the results given here show the first inactivation of fungal spores with TiO_2 photocatalysis and real well water at CPC scale, and therefore, are worth including in this thesis.

Abstract

Agriculture, especially intensive agriculture, is often plagued by phytopathogenic fungi like *F. solani*. Due to fungal resistance to many substances and phytotoxicity or environmental problems of many disinfection agents, increasing effort is being made to develop new techniques, for phytopathogen control. It has recently been shown that *Fusarium*, especially *F. solani,* which is pathogenic to plants and humans, is susceptible to small-scale solar photocatalytic disinfection with TiO_2 in distilled water.

This work shows results of disinfection of water contaminated with *F. solani* spores in a 14-L CPC solar photoreactor. With optimized TiO_2 concentrations and flow conditions, high disinfection yields (~99%) were possible, not only for distilled, but also for real well water. Furthermore, we present the design of the first TiO_2-based pilot plant for disinfection of phytopathogens. This design consists of various treatment units and improved CPC modules, adjusted for photocatalytic disinfection of resistant microorganisms like fungi.

Introduction

The high demand of water in agriculture draws growing attention to sustainable water resources. Water used or reused in agriculture has to be disinfected to remove any phytopathogenic microbes [Gomez, 1993 and 2003; Núñez *et al.,* 2006]. Spores of phytopathogenic fungi are especially resistant to many environmental factors [Beyer *et al.,* 2004], easily spreading in traditional plantations and especially in soilless cultures where diseases are often waterborne, and reach the plants in the nutrient solution [Gomez, 1993 and 2003; Runia, 1994; Runia and Boonstra, 2001]. Our target for disinfection, the filamentous fungus *F. solani*, for example, is found in soil everywhere [Summerell *et al.,* 1993; Sangalang *et al.,* 1995] and even in hospital water distribution systems [Anaissie *et al.,* 2001].

Solar photocatalytic disinfection with TiO_2 takes advantage of hydroxyl radicals produced by the semiconductor surface in contact with the water phase under $\lambda \leq 390$ nm solar irradiation. Hydroxyl radical generation is subdivided into various reaction steps and is accompanied by co-reactions depending on the catalysts surface and the molecules in the water, as fully described in the literature [Hoffmann, 1995; Herrmann, 1999; Augugliaro *et al*., 2006]. As the radicals generated are highly reactive, especially the $^{\bullet}OH$ radicals, TiO_2 has already been used for hazardous chemicals in water in many different applications [Malato *et al.,* 2002 a, b; Blake, 2001; Herrmann, 2005].

Disinfection with TiO_2 was first reported in the eighties for the Gram-positive bacteria *Lactobacillus acidophilus*, the Gram-negative bacteria *E. coli*, and the yeast *S. cerevisiae* [Matsunga *et al.,* 1985, 1988]. Very recently, disinfection of water containing pathogenic *C. parvum* oocysts, which are very chlorine resistant [Méndez-Hermida *et al.*, 2007] and *Fusarium sp.* spores have been reported in distilled water in the laboratory [Sichel *et al.,* 2007 a; Lonnen *et al.,* 2005]. Although in the beginning the interaction of the catalyst and the cell wall was neglected [Matsunga *et al.,* 1985, 1988], recent literature explains the disinfection process by lipid peroxidation of the cell wall [Gumy *et al*., 2006 b] and later lesions of microbial cell walls and leakage of cell content to the media [Nadtochenko, 2004; Kiwi and Nadtochenko, 2004; Sunada *et al.,* 2003; Saito *et al.,* 1992]. In most of these cases, the TiO_2 probably sticks to the microbial cell wall where the radicals generated

produce such lesions, as observed for disinfection of certain *Fusarium* spores with TiO_2 slurry [Sichel *et al.,* 2007 a].

Our previous work has demonstrated the susceptibility of *F. equiseti, F. oxysporum F. anthophilum, F. verticillioides*, and *F. solani* to disinfection with TiO_2 slurry under solar irradiation in 250-mL flasks in distilled water [Sichel *et al*., 2007 a]. However, the process has to be scaled up to be of interest for application, especially in the field of water treatment for reuse and irrigation in agriculture. Unfortunately, upscaling is always bound to meet with obstacles of different flow regimes, irradiation conditions, oxygen supply, etc. Therefore, recent contributions have been working on photocatalytic water disinfection using pilot plant solar CPC reactors [Rincón and Pulgarin, 2005 a, b; Fernandez *et al.,* 2005; Sichel *et al.,* 2007 b, c], but none of these have been for the disinfection of water with phytopathogenic fungi for reuse in irrigation. *F. solani* was chosen for this study based on our previous experience, due to its resistance to the disinfection process, and because it is so ubiquitous.

This contribution presents the results of solar photocatalytic disinfection of water contaminated with *F. solani* spores in a 14-L CPC reactor. The efficiency of the solar photocatalytic process is experimentally demonstrated in 5 h of treatment of distilled water and real well water. The effects of catalyst concentration and flow rate of the treated water are analyzed. The catalyst concentration was adjusted to the CPC-tube diameter and real well water. The design of a pilot treatment plant for disinfection of reused irrigation water in greenhouses based on these experimental results is presented.

Materials and Methods

Fungal strain and quantification: The wild strain of *F. solani* came from soil cultures in Almería (southern Spain). Fungal colonies were transferred to agar sporulation media, containing potassium chloride and kept at 25ºC for 15 days to produce microconidia, macroconidia and chlamydospores. The same strain and sporulation method had been used before [Sichel *et al.,* 2007 a, b]. The spores were washed with sterile water from the mycelia and agar. The resulting suspension was centrifuged at 2000 g for 10 min and washed three times with sterilized, distilled water. The spore concentration was determined

with a counting chamber (improved Neuenbauer). Then the spore suspension was diluted into the reactors. Depending on the fungal spore production on the agar plates, the initial spore concentration of the experiment varied from one day to the next between 0.3-1 x 10^3 CFU/mL.

The reduction in fungal concentration was measured by plate counting the decrease in cultivable fungal spores (CFU/mL). 50-250-µL of the samples were plated out on acidified malt agar (Sigma Aldrich, USA). Where fewer than 10 colonies per plate were observed, 500 µl of the sample were plated to reach a detection limit of 2 CFU/mL. Each measurement was made in triplicate, with three sample inoculations on three plates. After 2 days of incubation at 28ºC in the dark, the fungal colonies were counted. The measurements were analyzed by one-way ANOVA with $P<0.05$, Confidence > 95% (Origin v7.0300, OriginLab Corp., Northampton, USA).

Solar compound parabolic collectors: All experiments were performed under natural solar radiation at the Plataforma Solar de Almería, Spain, located at 37º84' N and 2º34' W. The solar CPC reactors were designed and built by AO SOL (Lisbon, Portugal) [Collares-Pereira *et al.,* 2006]. The system (**Figure V.1**) consists of two borosilicate glass tubes (id=4.64 cm) placed in the focus of CPC reflectors. Glass tubes and CPC collectors are held by aluminum frames mounted on platforms tilted at the local latitude of 37°. The glass tubes are connected in series so that water flows directly from one to another and finally into a tank. A centrifugal pump (20 Watts, Panworld, Spain) then returns the water to the solar collector. The tank has an opening on top into which contaminated water is poured. The treated water is later recovered by opening the outlet valve.

The irradiated collector surface is 0.42 m^2 and 4.7 L of the total photoreactor volume are illuminated. CPC reactors are often used for solar photocatalytic water treatment due to their high capacity for providing homogenous solar UV irradiation for photocatalysis [Malato *et al*., 2002 a, b]. The fungal suspension was left recirculating for 15 min in the dark reactor (adaptation and homogenization time). Then the first sample was taken and the reactor was exposed to solar irradiation. All samples were taken in 50 mL-tubes. The

first sample was kept in the dark at room temperature and analyzed again at the end of the experiment to determine the decrease in fungal concentration in the dark.

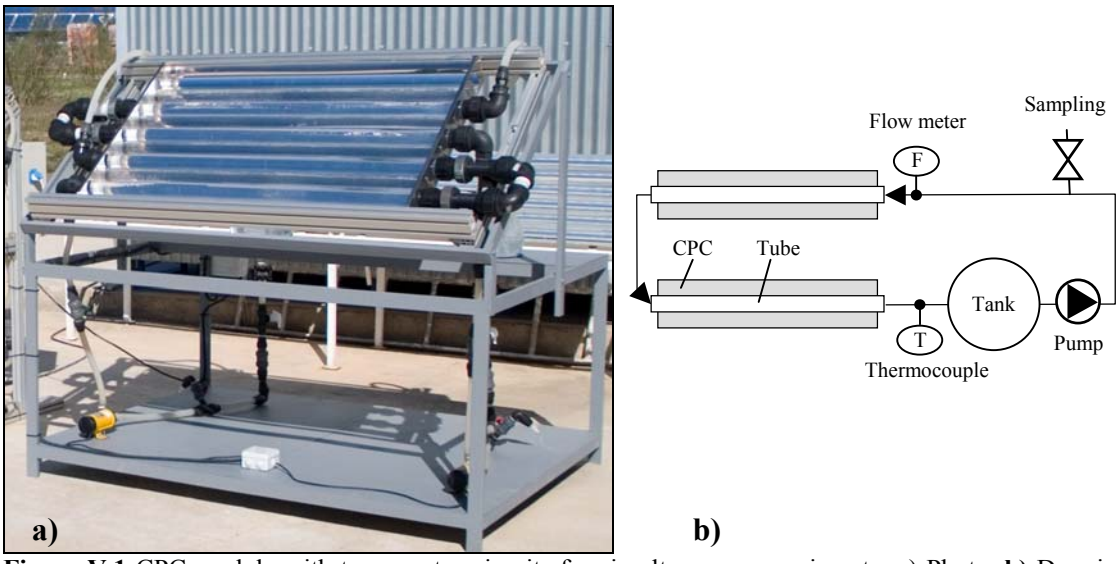

Figure V.1 CPC module with two reactor circuits for simultaneous experiments. **a**) Photo. **b)** Drawing of one reactor circuit with CPC collector, pump, sampling valve, flowmeter, thermocouple and tank.

The water temperature in the reactor, which was monitored but not controlled, did not exceed 35°C in any of the experiments. At these temperatures, fungal viability is not affected [El Halouat and Debevere, 1997; Samapundo *et al.,* 2007].

The experimental setup allowed two experiments to be performed simultaneously in twin solar CPC reactors installed at Plataforma Solar of Almería. The simultaneous experiments compared different catalyst concentrations and also the action of bare sunlight versus sunlight and catalyst. All experiments were repeated three times. As disinfection conditions can vary significantly on different days due to changes in irradiation, fungal spores and temperatures, the reactor experiments are not presented as average of the three repetitions, but as one representative experiment averaging the three repetitions. Each graph shows the simultaneous experiments. Normalized graphs in $\ln(c/c_0)$ are also presented to compare experiments performed on different days or with varying initial spore concentration. Solar UV-irradiance was measured with a global UV radiometer in the range of 295-385 nm, also mounted on a platform tilted 37° (Model CUV3, KIPP&ZONEN, the Netherlands). The average solar UV irradiance during the experiment is given in W/m^2.

Solar bottle reactor: The experiments were performed in 250-mL DURAN-Glass (Schott, Germany) reactor bottles. Glass covers (Schott) were used instead of plastic lids, to allow the solar radiation to enter the bottle reactor from all directions. An inner diameter of 50 mm and irradiated surface of (πr^2=19.6 cm^2) were considered. The bottles were stirred with magnetic stirrers at 100 rpm during the experiment. All experiments were performed in triplicate at the PSA. Solar photocatalytic and solar-only disinfection tests were carried out simultaneously, and the control bottles (one with and another without TiO_2) were stored under the same conditions, but in the dark. The fungal spores were added to the total 200-mL volume of water and left for 15 min in the dark (adaptation and homogenization time). Thereafter, the TiO_2 slurry catalyst was added and the bottle reactors were exposed to natural solar irradiation.

Well water: These studies were also carried out using natural well water so that the chemical inactivation environment would be similar to irrigation water which may have phytopathogenic fungi. The water was from an approx. 200-m well located at the PSA. Consequently, distilled water was used as a reference. **Table 5.1** (chapter 5) gives averaged physico-chemical parameters of the well water during the experiments, where it may be observed that this water has a higher carbonate content than usual.

The presence of other fungal species was not detected applying the same malt agar detection method as for the experiments. Water was analyzed for anions by ion chromatography (IC) with a Dionex DX-600 system and for cations with a Dionex DX-120 system. TOC and TC were analyzed in a Shimadzu TOC-5050 a analyzer. The pH was measured by a pH electrode (WTW, Germany). Turbidity was measured with a laboratory turbidimeter Model 2100N (Hach, USA).

Results and Discussion

Solar bottle reactors with distilled water: Recently, it has been shown that the *Fusarium* genus, and in particular, *F. solani*, is susceptible to solar photocatalytic disinfection with TiO_2 under laboratory conditions, in small volumes (250 mL) of distilled water [Sichel *et al*., 2007 a, b]. Disinfection at the 200 mL scale effectively reduced fungal spores by 3 logs to the detection limit within five hours (**Figure V.2**). Both represented experiments, solar

and solar photocatalytic disinfection (20 mg/L of TiO_2), where performed in parallel at high solar UV-A irradiance (average 40 W/m^2). Only solar photocatalysis was able to reduce the fungal spores 3 logs, to its detection limit. The dark experiment with TiO_2 does not show any inactivation of fungal spores at all.

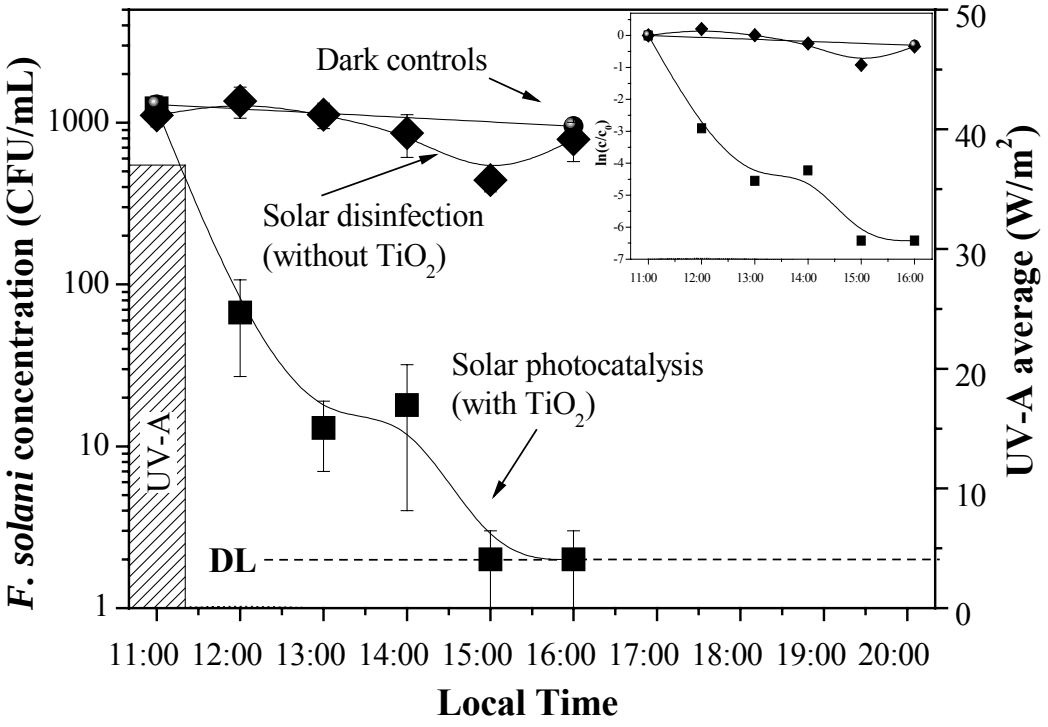

Figure V.2 Exposure of *F. solani* to natural sunlight in the presence of 20 mg/L TiO_2 (–■–) and in the absence of TiO_2 (–♦–). Dark control with TiO_2 (20 mg/L) (–●–). Dotted line represents temperature and column average irradiance during the experiment irradiated by sunlight. DL is the detection limit for the experiments, 2 CFU/mL. Inset: same experiment represented as ln (c/c_0).

The results shown in **Figure V.2** are similar to previous findings, but with lower TiO_2 concentrations: 20 mg/L instead of 35 mg/L [Sichel *et al*., 2007 a]. 35 mg/L was calculated to be the optimal concentration of TiO_2 P25 slurry for the bottle reactor used [Sichel *et al*., 2007 a; Fernández-Ibáñez *et al.,* 1999]. In fact, this result shows that disinfection in bottle reactors is very good even if TiO_2 concentrations are below the maximal yield concentration. The insert shows the same experiments normalized using ln (c/c_0). In this case, both graphs look very similar due to the original fungal spore concentration in all the bottle reactors.

Solar CPC reactors with distilled water: **Figures V.3 a, b** show *F. solani* spore survival in photocatalytic disinfection experiments and blank tests in distilled water in the CPC reactor in the dark and under sunlight. It may be observed that the initial spore concentrations are not the same in all experiments. Therefore, **Figure V.3 b** shows the three experiments normalized in $\ln(c/c_0)$. Survival in the CPC reactor in the presence of 50 mg/L TiO_2 in the dark shows that the reactor flow regime and the bare contact with the catalyst have no negative effect on the spore concentration.

In previous experiments with spores in this reactor at a flow rate of 10 L/min (results not shown), there was sedimentation of spores and TiO_2 particles. The fungus cell walls are expected to be negatively charged as are those of many other microorganisms in water with a few exceptions [Van Der Mei *et al.,* 1998]. At the slightly acid pH of 5.5 in the distilled water, the catalyst still had a positive surface charge [Fernández-Ibáñez *et al.,* 2000; 2003] and stuck to the cell wall of the fungus spores, increasing the weight of the aggregates, and the 10 L/min or lower flow was not enough to maintain them in suspension. Consequently, spores were lost in the reactor due to sedimentation of the catalyst and spores. The strong tendency of TiO_2 to adhere to the fungal spores has already been shown in our previous work [Sichel *et al.,* 2007 a], in which TiO_2 completely covered the spore surface during the 5-h experiment. This sedimentation effect (**Figure V.3 a**) was completely avoided simply by increasing the reactor flow rate to 20 L/min so that all spore inactivation under sunlight in presence of TiO_2 may be attributed to photocatalytic disinfection, not to sedimentation or other losses in the dark.

The blank test under solar irradiation shows the effect that bare sunlight has on the survival of the fungal spores. An average solar irradiation of 22.8 W/m^2 during the experiment reduced the fungal concentration from 2440 CFU/mL to 500 CFU/mL (0.7 log). The real improvement of the TiO_2 catalyst over solar-only disinfection in the reactor may be better understood through an analysis of their respective modes of action. Although both processes rely on solar energy for disinfection, TiO_2 has a completely different mode of action [Nadtochenko, 2004; Kiwi and Nadtochenko, 2004; Sunada *et al.,* 2003; Saito *et al.,* 1992]. While the TiO_2 attaches to the spore cell walls and oxidizes lipids and proteins, the

solar UV light enters the cell and causes oxidative stress after absorption by intracellular chromophores [Favre, 1977; Moan and Peak, 1989; Wondrak *et al.,* 2006].

In the 250-mL bottle reactors (**Figure V.2**), there was a 3-log decrease in *F. solani* concentration within similar treatment times, so apparently, the disinfection yield decreased in the CPC reactors (1.7 log), even though the solar UV-A energy per unit of volume was approximately 30% higher in the CPC reactor than in the bottle reactor (**Eq. V.1** and **V.2**):

$$Q_{UV}: Bottle: \frac{\overline{UV} \cdot A_i \cdot t}{V_t} = \frac{40W/m^2 \cdot 0.0020m^2 \cdot 21600s}{0.0002m^3} = 8.64\frac{kJ}{L} \quad \textbf{(Eq. V.1)}$$

$$Q_{UV}: CPC: \frac{\overline{UV} \cdot A_r \cdot t}{V_t} = \frac{22.8W/m^2 \cdot 0.41m^2 \cdot 18000s}{0.014m^3} = 12.02\frac{\mathrm{kJ}}{\mathrm{L}} \quad \textbf{(Eq. V.2)}$$

Where Q_{UV} is the accumulated solar UV energy per unit of treated water volume, $\overline{UV}$ is the average UV irradiance received in the reactor during the experiment. A_r is the illuminated area of the reactor, V_t is the total water volume for disinfection and t the total treatment time.

This decrease can be explained in the following way: Increasing the total treatment volume, the disinfection is carried out in a solar reactor system of several liters. Nevertheless the CPC and tank design brings the disadvantage of needing a tubing system. The tubing system and connections add dark parts to the reactor system.

In our CPC reactor, only 4.7 L of the total 14-L volume could be illuminated. Agitation of the water is also needed for homogeneous mixing of the catalyst, and good contact between fungal spores and catalyst. But the dark parts of the reactor causes the total irradiation time for the fungal spores to be shorter than in the completely illuminated systems. The lower average UV-A irradiance would also probably contribute to the explanation for the slower decrease in fungal concentration in CPC reactors compared to bottle reactors. This effect was even stronger in these CPC experiments due to the lower irradiance of 22.8 W/m^2 compared to 40 W/m^2 during the bottle reactor experiment. But 20 W/m^2 average solar UV

irradiance has also been enough to reduce *Fusarium* spores 3 logs in bottle reactors and in the presence of TiO_2 [Sichel *et al*., 2007 a].

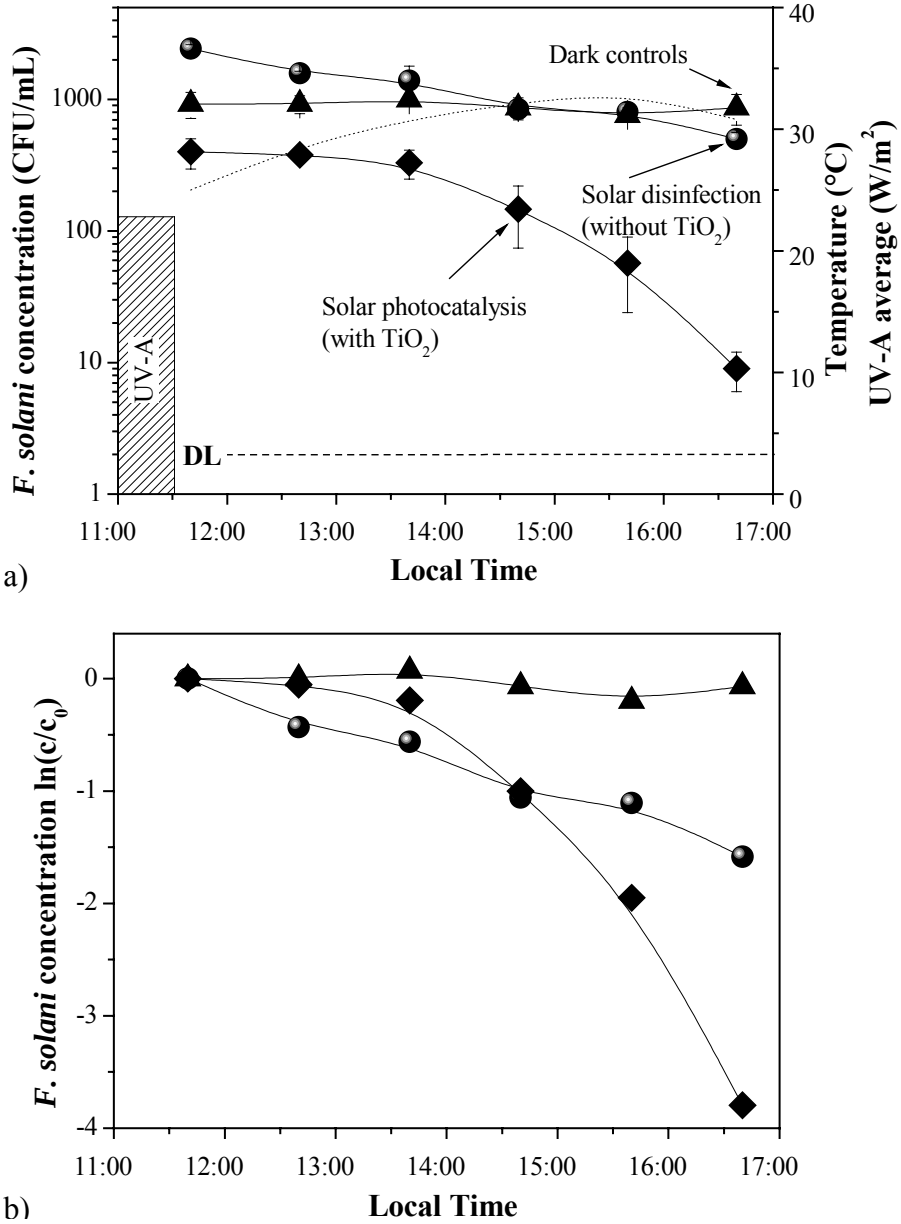

Figure V.3 a) Exposure of *F. solani* to natural sunlight with 50 mg/L TiO_2 (–♦–) without TiO_2 (–●–) and 50 mg/L TiO_2 in the dark (–▲–). Experiments were performed in distilled water in two CPC reactors at a flow rate of 20 L/min. Each point represents the average of samples taken in one representative experiment; the bars show the statistical error that yields a 95% confidence level. The dotted line shows temperature, and column the average solar UV-A irradiance during the experiments. **6.3 b**) The same results in $ln(c/c_0)$.

Nevertheless, even with lower reduction of total fungal spores, the use of CPC systems still represents an important improvement over the 250 mL bottle reactors. The photocatalytic water disinfection technique is only of interest for irrigation water in large scales of liters or even cubic meters. This is possible in CPC plants, because the process can be scaled up just by increasing the number of the CPC modules. One possible way to increase treatment volume and disinfection yield of our CPC plants is shown in **Figures V.5, V6.6**.

Solar CPC reactors with well water: Figures **Figures V.4 a, b** show experiments performed with TiO_2 in real well water. Both graphs show the the effect of catalyst concentration in the CPC system. TiO_2-based disinfection was achieved at all tested concentrations, 30 mg/L, 50 mg/L and 70 mg/L. In **Figure V.4 a**), the disinfection yield can be observed to increase with increasing concentration from a 0.9-log-reduction at 30 mg/L to a 1.5-log reduction at 50 mg/L. On the contrary, when the concentration is further increased from 50 mg/L to 70 mg/L, disinfection decreased from 1.5 to 1.3 log (**Figure V.4 b**).

This behaviour can be explained by TiO_2 being a light absorbing and scattering semiconductor particle. The generation of $^{\bullet}OH$ radicals during the process, increases with increasing catalyst concentration until more light is inhibited by shadowing particles than is used by the TiO_2 [Herrmann, 1999]. The optimal concentration can be theoretically and empirically estimated by the optical path length in the CPC collectors (d_i=4.64 cm), assuming that its optimal point is reached when 99% of the incoming light (in the range of the catalyst excitation spectrum) is absorbed. In this case, the estimated optimal concentration is 41.3 mg/L [Fernández-Ibáñez *et al.,* 1999, Sichel *et al.,* 2007 a].

The 30 mg/L, 50 mg/L and 70 mg/L concentrations tested show that, in fact, disinfection follows the expected tendency for the concentration, reaching the best disinfection yield with 50 mg/L. As mentioned in the Materials and Methods Section, only two simultaneous experiments could be performed in one day. Therefore both graphs (**Figure V.4 a, b**) represent two experiments performed simultaneously. Each graph shows the experiments performed on a different day but with similar average solar irradiances of 22.6 W/m^2 in **Figure V.4 a** and 22.2 W/m^2 in **Figure V.4 b**. The similar irradiances explain the similar

reduction at 50 mg/L on different days. The water temperatures shown in the graphs are also very similar at below 30ºC. Therefore, water temperature is not thought to have a significant influence on the results.

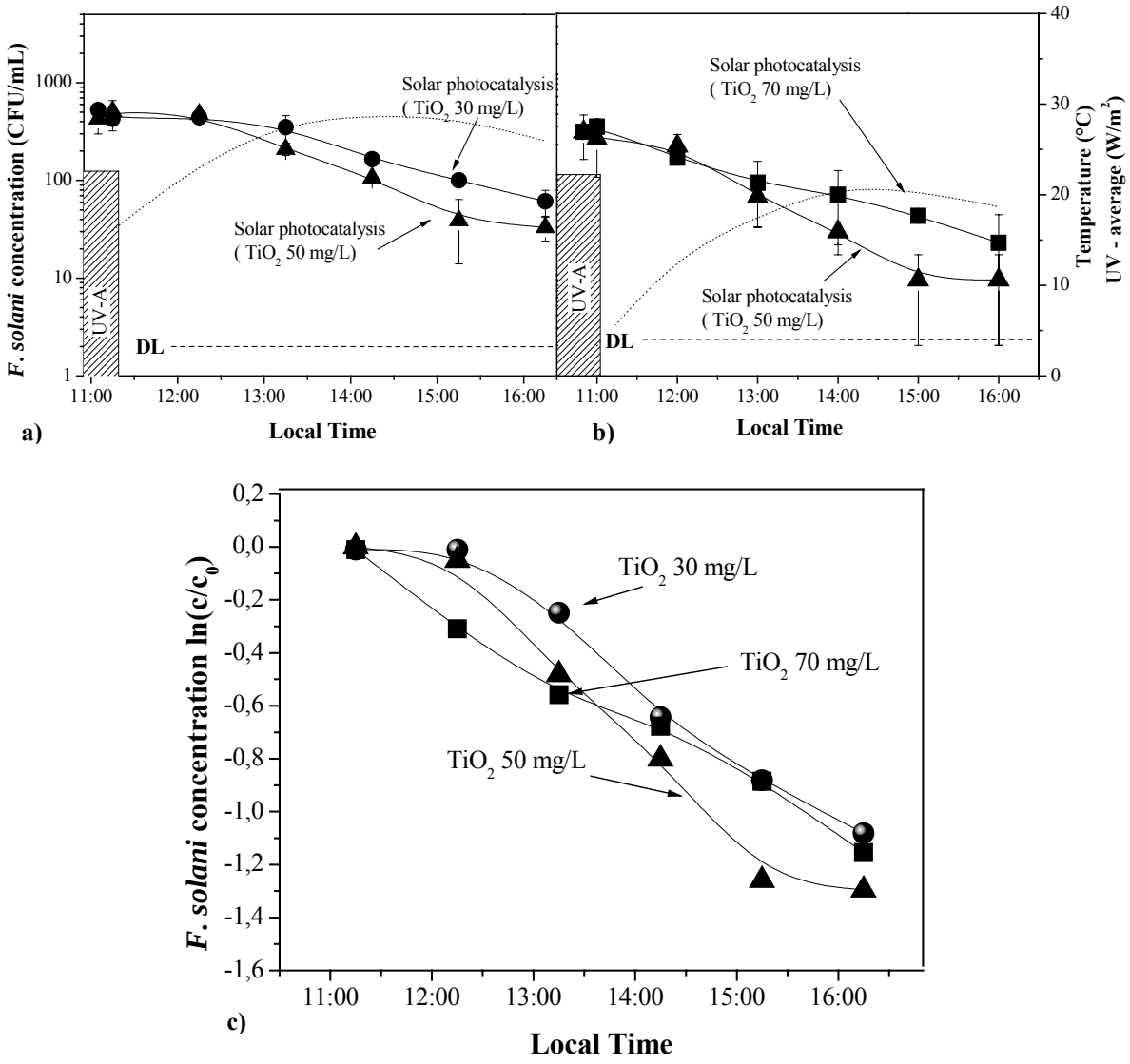

Figure V.4 a, b) Exposure of *F. solani* to sunlight in the presence of TiO_2 at concentrations of 30-70 mg/L in well water in CPC reactors. The TiO_2 concentrations were 30 mg/L (–●–), 50 mg/L (–▲–) and 70 mg/L (–■–). Flow rate was 20L/min in both figures. Each point represents the average of samples taken in one representative reactor experiment; bars show the statistical error that yields a 95% confidence level. Dotted line represents temperature and column average irradiance during the experiments. **V.4 c)** Comparison of the average of both 50 mg/L TiO_2 experiments and the 30 mg/L and 70 mg/L experiments performed on different days in $\ln(c/c_0)$.

Figure V.4 c is shows a comparison of all disinfection yields normalized in $\ln(c/c_0)$. The decrease in fungal spores with a 50 mg/L TiO_2 concentration is the average of the two experiments performed on different days. **Figure V.4 c** with 30 mg/L shows the typical solar-only disinfection lag phase [Sichel *et al*. 2007 c], which still has an impact at low TiO_2 concentrations, when not much solar light is absorbed by the catalyst particles. Then, after 1-2 hours, fungal concentration starts to decrease, and the final disinfection yield is almost the same as with 50 mg/L TiO_2.

With 70 mg/L, damage by the irradiated TiO_2 takes place from the beginning of the reaction on then the process slows down. In these experiments, the best concentration was 50 mg/L, showing the fastest fungal inactivation with the lowest spore concentration at the end of the experiment.

In turbid water, the optimal concentration of TiO_2 is lower, due to additional shadowing caused by light absorption and scattering of the suspended material. The well water used had a low turbidity of 5 NTU, therefore, the disinfection yield was lower for experiments in real water compared to distilled water. From a 1.7-log reduction in distilled water (**Figure V.3**) under slightly higher average irradiance of 23.3 W/m^2 with 50 mg/L TiO_2, the disinfection yield fell to a 1.5-log reduction.

Enhanced CPC pilot plant design: Based on the positive, but challenging experiments in the existing CPCs, the following CPC disinfection modules and pilot plant were designed. **Figure V.5** shows two CPC collector modules, connected in series from one to the next. These two modules will be integrated in the pilot plant in **Figure V.6**, currently under construction. These CPC modules combine several advantages for scaling up the process in the experiments presented (**Figure V.2-V.4**). The 90 W pump will provide a turbulent flow regime with a 30 L/min flow rate (Reynolds 16.000). Future experiments in the pilot plant designed are expected to show that flow rates of 30 L/min significantly increase the *F. solani* solar photocatalytic disinfection process, due to better homogenization, particle suspension and increased oxygenation.

Another factor which improved the new design was the increase in ratio of illuminated to dark (piping and tank) reactor volume. In the CPC used in these experiments, with only 4.7 L of irradiated volume out of its 14-L total volume, only 33.6% of the total water volume is illuminated. This yields one third of the total illumination time, compared with the enhanced system. An important improvement in the new plant design is therefore the increase in total illuminated volume. Of the 60-L total volume per batch, 38 L (63%) will be illuminated during the process. For this, the illuminated surface is increased to 3.64 m^2 and the piping retains the least possible volume (approx. 5 L). The borosilicate tubes used are the same diameter (50 mm d_e) to avoid changing the optimised catalyst concentration. Nevertheless, they will be thicker (2.5 mm) to make the system more robust.

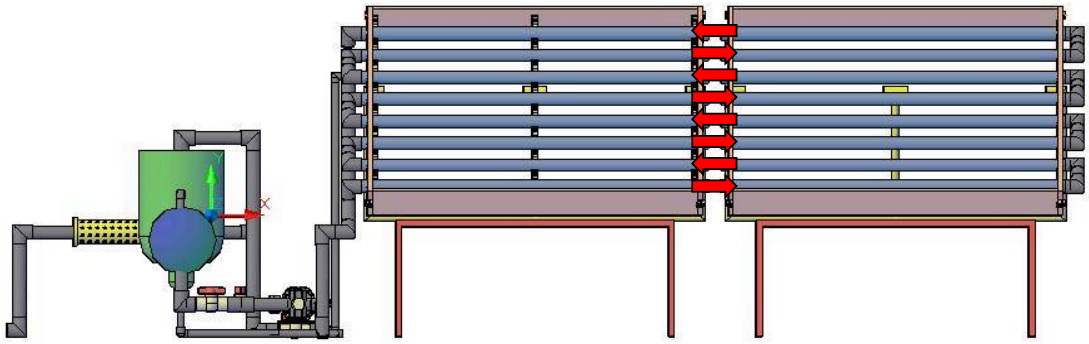

Figure V.5 CPC reactor designed for disinfection experiments with conical tank and pump.

The pilot plant designed not only has two CPC modules, but also a pre and post treatment unit. The first filter at the entrance of the pilot plant is optional to reduce suspended solids in the water which could cause turbidity and competition for adsorption of the catalyst and the $^{\bullet}OH$ radicals produced. After the first filter unit, the water is stored in a tank to ensure continuous inflow to the CPC system and to increase the total volume of treated water. Pre-suspended TiO_2 powder is added in the tank. Then batch disinfection takes place in the water recirculating through the two CPC modules and the tank. After reaching the target disinfection level, the TiO_2 suspension is let out to the second tank, the sedimentation tank. The sedimentation unit is installed to recover the catalyst and to reduce the load of solids going through the last filter unit. pH in the tank can be varied to accelerate of P25 TiO_2

particle sedimentation at neutral pH [Fernández-Ibáñez *et al*., 2003]. The TiO_2 recovered can be recycled, depending on the water qualities and how contaminated the catalyst surface has become. Then the water treated is passed through a filter unit and can be used for irrigation.

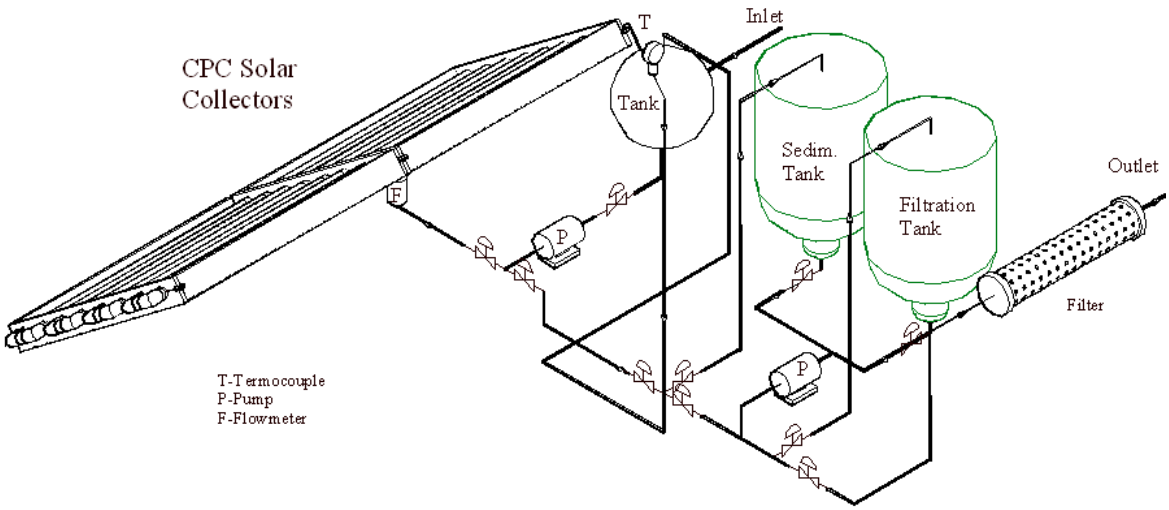

Figure V.6 Pilot plant design with CPC modules, catalyst sedimentation tank and filters for post- treatment.

The treatment times can be varied according to the solar irradiation by solar-sensitive control devises. Dissolved oxygen, pH and temperature are monitored throughout the treatment. The complete treatment unit only occupies an area of 5 m^2 and has a treatment capacity of 60-120 L/day depending on weather and water conditions. The installation costs are low, as the aluminum and borosilicate glass tubes in the collector are already standard in photocatalytic water treatment reactors (approx 8 euros/borosilicate tube, Vidrasa, Spain).

The other component parts of the treatment unit are even the same as used in traditional water treatment and can be easily replaced. The only operating costs are the centrifugal pump, and the catalyst. TiO_2 is approximate 17 euros/kg [Degussa, Germany 2008], which would lead to a cost of 0.85 €/m^3 of treated water without recycling the catalyst.

Conclusions

- Solar photocatalytic disinfection can be applied to various liters of distilled water and well water in CPC reactors. The use of real well water with low turbidity slightly reduces the disinfecting action of TiO_2.

- Scale-up of the photocatalytic process requires the flow regime, irradiated volume, catalyst concentration, etc., to be adjusted. The optimized experimental concentration for water with low turbidity is still very similar to the one theoretically calculated: 50 mg/L for a 4.6-cm inner tube diameter.

- After testing the existing CPC reactor under different engineering conditions, a TiO_2 disinfection pilot plant has been designed with an increased illuminated volume and flow rates. This pilot plant will consist of two CPC reactor modules and pre-and pos-treatment units. Depending on the water quality, the catalyst can recycled by sedimentation. Under good irradiation conditions, this plant will be able to treat 60-120 L/day.

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VI. Conclusions and Outlook

The combination of the work presented shows great potential for the application of solar photocatalytic disinfection treatment of water, especially for agricultural use. This potential has not been reported before, for any fungus or for agricultural applications. The diversity of process conditions tested in this work, water sources, process conditions and different microorganisms susceptible to the different methods, shows that the field of application is wide and not restricted to special water sources or very high radiation.

The inactivation of the different species of resistant *Fusarium* spores and spore types is a reason for special confidence in this process, showing its generalized high disinfection potential. The use of solar energy and nontoxic, natural substances makes it a sustainable and ecologic alternative to traditional disinfection agents. Using solar energy also has the advantage of its applicability in rural areas, by combining the CPC modules with solar cells for driving pumps. The system's modularity allows successive scaling up of disinfection installations, by adding more modules or raising the volume per module.

Further work will surely lead to its successful application in greenhouses for water disinfection for both TiO_2-based photocatalytic disinfection and synergetic disinfection with H_2O_2 and sunlight. The second has the advantages of homogeneous processes. While TiO_2 has to be separated from the water for its reuse and to avoid high loads of solids in the water, H_2O_2 reacts to nontoxic concentrations during the process and can remain in irrigation water. The field of application for both processes is wide, from water disinfection to disinfection of seeds and possibly even in the medical field for surface disinfection of objects or treatment of skin diseases.

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VIII. Glossary

a	Aperture of solar collector (Eq. 6) (m^2)
AFM	Atomic force microscopy
A_r	Illuminated reactor area
AOP	Advanced Oxidation Process
ATCC	American Type Culture Collection
ATR-FTIR	Attenuated total reflection Fourier transform infrared spectroscopy
C	Concentration of bacteria or fungi (CFU/mL)
C_0	Initial concentration of bacteria or fungi (CFU/mL)
CF	Concentration factor of a solar collector (adimensional)
CFU	Colony forming units
CLA	Carnation Leaf Agar
CPC	Compound parabolic collector
CWD	Coffee wilt disease
DBP	Disinfection by-products
DBPPs	Disinfection by-products precursors
DDT	Dichloro-diphenyl-trichloroethane
DL	Detection limit (CFU/mL)
DMI	Demethylation inhibitors
DMIs	Demethylation inhibitors
Dose	$Dose = \int_{t1}^{t2} Irradiance \cdot dt$; (J/m^2)

DSSR	Double Skin Sheet Reactor
DWHH	Deutsche Welthungerhilfe
e^-	Electron
EDT	Effective disinfection time (h)
E_G	Band gap energy (eV)
EPA	US-Environmental Protection Agency
f. sp. (pl. f. spp.)	*formae speciales*
FAO	Food and Agriculture Organization of the United Nations
FRAG	Fungicide Resistance Action Committee
h	Plank constant
h^+	Electrone hole (vacancy)
IEP	Isoelectric point
Irradiance	Irradiant energy per unit of time and surface area (W/m^2)
LB	Luria Bertani Agar
LIP	Labile iron pool
MeBr	Methyl bromide
NTU	Nephelometric turbidity units
PBT	Persistent, bioaccumulative, and toxic pollutants
PET	Polyethylene terephthalate
PSA	Plataforma Solar de Almería
PTC	Parabolic-trough collector
PVC	Polyvinyl chloride
PZC	Point of zero charge (adimensional)

θ_a	Semiangle of acceptance for solar collectors (rad)
Q_{90}	Accumulated UV energy per unit volume (Q_{UV}) necessary to reduce the concentration of viable bacteria by 90% (J/L)
Q_{UV}	Global radiant UV energy accumulated per unit of volume (J/L)
r	Radius of tube (Eq. 6) (m)
ROS	Reactive oxygen species
SODIS	Solar (batch) Disinfection process
TFFBR	Thin Film Fixed Bed Reactor
THM	Trihalomethanes
TOC	Total organic carbon (mg/L)
ν	Frequency (s^{-1})
USDA	United States Department of Agriculture
UV	Ultraviolet light
UV-A	Ultraviolet-A, wavelength range 320-380 nm also called near UV or N-UV
UV-B	Ultraviolet-B, wavelength range 290-320 nm also called mid-UV or M-UV
UV-C	Ultraviolet-C, wavelength range 190-290nm, also called far-UV or F-UV
V_t	Total volume of the photo reactor (L)
WHO	World Health Organisation
λ	wavelength (nm)

IX. Scientific Contributions

Articles published in refereed (JCR) journals

Sichel, C., de Cara, M., Tello, J., Fernández-Ibáñez, P. 2008. Resistance of macroconidia and chlamydospores of *Fusarium equiseti* to solar photocatalytic disinfection. Journal of Plant Disease. Under review.

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