Faecal coliforms, faecal enterococci, *Salmonella* Typhi and *Acanthamoeba* spp. UV inactivation in three different biological effluents

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Abstract

Efficiency of UV-light to inactivate microbial indicators, *Salmonella* Typhi and *Acanthamoeba* spp. was studied in three different biological secondary effluents. Even though effluents differed in terms of their total suspended solids content, transmittance and particle size distribution, the UV-light dose required to fulfil WHO agricultural water reuse criteria was the same (30 mW·s/cm²), because the particle content with sizes >40 μm was similar and very small. Using this dosage, 3 log of *Salmonella* Typhi and faecal enterococci were also inactivated. To avoid faecal coliform and *Salmonella* Typhi photoreactivation, the UV dose had to be doubled and in the process 2.5 log of *Acanthamoeba* spp. were also inactivated. This is interesting because its presence in wastewater, pathogenicity and resistance to conventional disinfection processes has been reported in the literature. Additionally, it was found that the faecal coliforms' inactivation rate constant was the lowest one of all the bacteria studied (*Salmonella* Typhi and faecal enterococci), suggesting the limitation of this indicator when several kinds of pathogens are present, as is the case in developing countries.

Keywords: amoebae, UV disinfection, indicators, pathogen, risks assessment, wastewater

Introduction

In 2001, water-borne diarrheic diseases represented 13% of the 15 million deaths caused by all types of infectious diseases worldwide, 74% occurring in children under the age of 5 in the developing world (Hinrichsen and Robey, 2000). One of the main causes of diarrhoea is the consumption of crops irrigated with polluted water. It is estimated that at least 20 x10⁶ ha in 50 countries are irrigated using wastewater and that 10% of the crops consumed in the world are produced this way, although for some countries, such as Thailand, consumption may be as high as 90% (Carr et al., 2004; Scott et al., 2004). There are no estimates of the amount of crops produced using polluted water but it is reasonable to assume that the figures could be much higher than those presented for wastewater. This is of particular concern for developing countries because, on the one hand, higher concentrations of a wider variety of organisms (bacteria, viruses, protozoa, and helminth ova) are present in wastewater compared to developed countries' wastewater (Jiménez et al., 2001), and on the other hand, it is frequently cited in the literature that the behaviour of pathogens during disinfection is not properly modelled by common microbial indicators (Chang et al., 1985; Ashbolt et al., 2001).

Salmonella Typhi is one such pathogen usually present in wastewater produced in developing countries and in high concentrations. This bacterium is responsible for typhoid fever, a disease transmitted through the ingestion of polluted water or food which is very common worldwide. It is estimated that

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12.5 million people worldwide are infected annually. In Mexico alone, 7 481 cases of typhoid fever were reported in 2002; by comparison, there were 400 cases in the United States, 200 in the UK and 90 in France (WHO, 1998; CDC, 2001).

Protozoa are another group of micro-organisms contained in wastewater. The pathogenic enteric protozoa compromise the health of millions of people and contribute significantly to diarrhoeal disease morbidity. Their prevalence varies between 2 and 5% in developed countries while in developing ones it is 20 and 30% (Norhayati et al., 2003). It is currently perceived that *Cryptosporidium* (mainly) and *Giardia* (occasionally) are the only emerging protozoan pathogens, but there are many others in wastewater, depending on the local health situation. One example is *Acanthamoeba* spp. which is a non-faecal pathogenic protozoan found in a variety of environments and isolated from wastewater used to irrigate crops in Mexico (Maya, 2000). Its mode of infection is believed to be via skin lesions or through the respiratory tract rather than oral ingestion, occurring mainly in immuno-compromised individuals or young children. It can cause diseases in farmers if they come in direct contact with wastewater, as frequently happens in areas where non-treated wastewater is used for irrigation and in the general population when sprinkler irrigation is used or when children play in the open canals transporting wastewater. Moreover, wastewater and polluted soil have been described as pathogen reservoirs that pollute crops during cultivation by splashing or through wind-blown dust or by direct contact with fruits. Although rare, *Acanthamoeba* infections can cause death, as has been reported in the USA, Australia and Europe, which are countries with enough institutional capacity to track down the disease (Dreelin, 2005).

The sanitary importance of *Acanthamoeba* resides in its pathogenicity and resistance to conventional disinfection processes (Martínez and Visvevara, 1997). UV-light disinfection is an efficient method for inactivating bacteria, viruses and some

TABLE 1		
Analytical techniques used for physical and microbiological characterisation		
Parameter	Unit	Technique/Reference
Total suspended solids, TSS	mg/ℓ	Gravimetric, *Standard Methods* (1998), method 2540 D
Transmittance	%	Photometric
Filtered transmittance (0.45 μm)	%	Photometric
Particle size distribution, PSD	μm	Coulter principle, *Standard Methods* (1998), method 2560 B
Faecal coliforms, FC	CFU/100 mℓ	MF, *Standard Methods* (1998), method 9222 D
Faecal enterococci, FE	CFU /100 mℓ	MF, *Standard Methods* (1998), method 9230 C
Salmonella Typhi	CFU /100 mℓ	MF, *Standard Methods* (1998), method 9260 D
MF: Membrane filtration		

protozoa without producing by-products, as is the case with chlorine (Dodds et al., 1999; Linden et al., 2002). However, its effects on *Acanthamoeba* have been rarely studied (Maya et al., 2003). The required UV dose for an effluent depends on the water quality, the previous treatment process and the targeted micro-organisms. UV-light is considered to be an interesting option for disinfecting effluents intended for agricultural reuse because it avoids the presence of organochlorides in aquifers recharged with the wastewater excess used to irrigate. This is important because it has been reported that aquifers recharged in this way are also used as a drinking source, for instance in Mexico, Peru and Thailand (Foster et al., 2003; Jiménez and Chávez, 2004). Unfortunately, even though there is ample literature on UV-light disinfection, this technology has scarcely replaced chlorination. Most of the literature focuses on the inactivation of microbial indicators such as faecal coliforms (in most of the cases), faecal enterococci and *E. coli* (Jijnen et al., 2006) or other micro-organisms of interest in the developed world. Knowledge of the effect of UV-light on pathogens of interest to the developing world, such as *Salmonella* Typhi and *Acanthamoeba* spp., is poor and so it would be useful to define its possible effects on these or other similar pathogens. Considering the abovementioned facts, this research evaluated UV-light inactivation of indicators and other pathogens usually contained in wastewater intended for agricultural reuse in concentrations common for developing countries. Because UV-light disinfection efficiency depends on the quality of the effluent (Madge and Jensen, 2006), three different types of effluents from widely used biological processes were studied. The objectives were to:

- Determine the effect of the type of effluent (in terms of total suspended solids (TSS) content, transmittance and particle size distribution) on UV-light efficacy
- Analyse the effect of using a UV-light disinfection dose which fulfils WHO criteria doses for faecal enterococci, *Salmonella* Typhi and *Acanthamoeba* spp. as well as their disinfection kinetics parameters
- Evaluate the reactivation of faecal coliforms and *Salmonella* Typhi
- Analyse the effect of micro-organisms embedded in particles on disinfection efficiency. Additionally, the health risks caused by *Salmonella* Typhi on wastewater disinfected according to the WHO guidelines were determined.

Experimental

Sampling

Wastewater from a treatment plant (WWTP) located in the National Autonomous University of Mexico was selected for this study. This plant treats 40 ℓ/s of municipal wastewater coming from the university campus and a nearby residential neighbourhood. It has three biological processes working in parallel: activated sludge, trickling filter and rotating biological contactors. The activated sludge (AS) process treats 20 ℓ/s at a 0.3 kg BOD/m³·d load, a 5 h hydraulic retention time with a mean cell residence time of 5 d. The trickling filter (TF) operates at 10 ℓ/s, has a hydraulic retention time of 0.6 h, a 50% recirculation ratio and a BOD load of 0.5 kg/ m³·d. Finally, the rotating biological contactor (RBC) has a flow of 10 ℓ/s, 1 h hydraulic retention time, 1.6 r/min rotational speed and operates at 10 gBOD/m²·d load.

For the disinfection tests, 4 ℓ of effluent were collected from the secondary clarifier of each biological process every week for 3 years at the same hour of the day and during the rainy season (May to July). The rainy season was selected as a sampling period as diarrheic morbidity rates reach their maximum values during this period, and therefore the pathogenic content in the wastewater is higher. Samples were taken in clean plastic containers and kept at 4ºC for their immediate analysis (less than 0.5 h).

Effluents characterisation and irradiation tests

The effluents were characterised physically and microbiologically following *Standard Methods* (1998) procedures (Table 1). The particle size distribution was determined, both in volume and in number, using the coulter principle. Particle size distribution in volume was calculated assuming that particles were spherical with a diameter equal to the logarithmic average of the particle diameters measured for each size range (Adin, 1998).

UV-light disinfection tests were carried out using a collimated-beam reactor, with a horizontally suspended low pressure mercury lamp projecting light perpendicularly to the sample surface. Each test was repeated at least 3 times, using a 50 mℓ continuously stirred sample (25 mm magnetic bar) in a 55 mm diameter sterile glass container. The mean intensity of UV-light applied was 0.22±0.02 mW/cm² and was calculated by measuring the irradiation intensity at the beginning and at the end of each experiment. Intensity was measured with an International Light Inc. Calibrated Radiometer, IL 1700 coupled with an SED240 #4817 detector placed at the same height as the sample. The detector measured light-wave lengths of between 200 and 320 nm. The amount of UV-light applied was estimated by subtracting the quantity absorbed by water calculated using Beer's law. The final dose applied (mW·s/cm²) was calculated using Eq. (1):

$$D = I \, x \, t \tag{1}$$

where:

I = Intensity of UV light (mW/cm²)
t = Exposure time (s)

To obtain different irradiation doses (between 5 and 120 mW·s/cm^2) the exposure time was varied during the experiments.

To measure the disinfection efficiency, conventional biological indicators (faecal coliforms and enterococci) were used and two specific pathogens (*Salmonella* Typhi and *Acanthamoeba* spp.) were deemed to be of interest because they are disease agents common to developing countries and are associated with the use of wastewater for irrigation, as explained earlier.

Faecal coliforms, faecal enterococci and *Salmonella* Typhi disinfection tests were carried out without adding bacteria strains to the wastewater, i.e., just considering the 'normal content' in the wastewater. This content resulted in 2 to 3 log orders of magnitude, higher than that found in developed countries (Jiménez et al., 2001; Tchobanoglous et al., 2003). Amoebae disinfection experiments were performed in 50 mℓ of filtered and sterilised activated sludge effluent inoculated with 200 µℓ of a concentrated suspension of amoebae (10^4 cysts/mℓ *Acanthamoeba* spp.). The suspension was prepared following the recommendation given by Maya et al. (2003) by inoculating the trophozoite form in an axenic culture and incubating it at 30°C over a period of 7 d to avoid culture contamination. The incubated suspension was then centrifuged at 2 500 r/min for 10 min. The *Acanthamoeba* spp. content in the sediment was then measured using a Neubauer chamber 0.1 mm in depth. The procedure was repeated until a 10^5 cells/mℓ trophozoite concentration in the culture was obtained. A similar process was conducted to prepare an *Acanthamoeba culbertsoni ATCC 30171* suspension used as a reference strain to confirm the identification of amoebae. Faecal coliforms, faecal enterococci and *Salmonella* Typhi content were measured (Table 1) using the methods proposed in *Standard Methods* (1998). *Acanthamoeba* spp. and *Acanthamoeba culbertsoni ATCC 30171* were analysed following an ex-cystation technique (Craik et al., 2000) that consists of determining the total quantity of cysts susceptible to be transformed into trophozoites under favourable conditions in a monoxenic culture (in which amoebae are grown in the presence of a single additional species).

Reactivation experiments

UV-light does not have a residual disinfection effect once the effluent leaves the UV photoreactor. This is a concern because many micro-organisms are able to recover rapidly from the damage caused by the process. Photoreactivation (photo repair) is produced by an enzyme activated with 300 to 500 nm wavelength energy, which is normally found in water exposed to sunlight. As a consequence, the number of viable bacteria may increase within minutes to hours after disinfection once an effluent is discharged into the environment (Zimmer et al., 2003). This is a cause for concern when treated effluents are not immediately used and are transported in open channels, as frequently happens with treated wastewater reused for irrigation. Under such conditions, it is important to apply a UV dose that can also prevent its reactivation, besides inactivating bacteria. This dose is always higher than the inactivation dose and needs to be determined for each type of micro-organism and effluent. To study photoreactivation, experiments were performed only using bacteria (faecal coliforms and *Salmonella* Typhi). Since the reactivation rate does not depend on the same physical characteristics of the effluents that are important to the UV-light disinfection efficiency, experiments were carried out using only the activated sludge effluent. For this purpose, a 50 mℓ sample was disinfected using different UV-light doses. The irradiated effluent was placed in sterile glass bottles and followed by reactivation in both sunlight and darkness conditions for 2 h (Liltved and Landfald, 2000). Each test was performed twice. The reactivation rate was calculated using Eq. (2) (Lindenauer and Darby, 1994):

$$\text{Log} \frac{N_r}{N_0} - \text{Log} \frac{N}{N_0} = \text{Log} \frac{Nr/N_0}{N/N_0} = \text{Log} \frac{Nr}{N} \qquad (2)$$

where:

N_0 = Micro-organism concentration before UV irradiation (CFU/100 mℓ)

N = Micro-organism concentration after UV irradiation (CFU /100 mℓ)

N_r = Micro-organism concentration after reactivation (CFU /100 mℓ)

The dose reduction factor (DRF) used to predict the increase in the UV inactivation dose to avoid photoreactivation (Lindenauer and Darby, 1994) was calculated using Eq. (3):

$$\text{DRF} = \frac{\substack{\text{UV dose required for a given log survival after} \\ \text{UV disinfection without photoreactivation}}}{\substack{\text{UV dose required to obtain the same log survival} \\ \text{with photoreactivation}}} \qquad (3)$$

Effect of embedded micro-organisms on the inactivation efficiency

The dose-response disinfection curve is specific for each type of micro-organism and effluent (USEPA, 1986). It has 2 regions. A 1st phase, where low UV-light doses inactivate the micro-organisms that are freely suspended in wastewater: this part of the curve is modelled by a first-order kinetics reaction. The 2nd phase of the curve is the tailing region. Under these conditions inactivation is slightly improved although UV-light doses are considerably increased. In this case, doses do not depend on the total number of micro-organisms but on the amount and size of particles, because micro-organisms are embedded in them and their numbers are difficult to determine. To estimate the UV-light dose needed, mathematical models using lumped parameters have been developed (USEPA, 1986). The lumped parameters commonly used are the TSS content, the unfiltered transmittance and the filtered transmittance. Once models are calibrated to a specific effluent, they become useful for designing full-scale UV disinfection facilities.

Experiments to evaluate the effect on the inactivation efficiency of the presence of micro-organisms embedded in particles were carried out using different TSS content as suggested by Andreadakis et al. (1999). The activated sludge effluent was used for these experiments because it had a higher TSS content and it was possible to prepare spiked samples by adding mixed liquor from the aeration tank to the effluent producing different TSS contents (20 to 100 mg/ℓ). Before irradiation, the TSS and the faecal coliform content were determined. The sample was then exposed to different UV-light doses (between 5 and 120 mW·s/cm^2) and then faecal coliform content was once again measured. To calculate the theoretical micro-organism content embedded in particles (Np) in the tailing region, Eq. (4) was used (USEPA, 1986). The TSS and the Np values used for such calculations were the mean values obtained for the different TSS ranges (0 to 15 mg/ℓ, 15 to 30 mg/ℓ, 30 to 45 mg/ℓ, 45 to 60 mg/ℓ and 60 to 100 mg/ℓ).

Parameter	Activated sludge (AS)	Rotating biological contactors (RBC)	Trickling filter (TF)
Total suspended solids, mg/ℓ	31±10	9±2	12±1
Transmittance, %	55±7	75±9	65±8
Filtered transmittance, %	74±3	85±3	79 ±3
Mean particle size, μm	12±8	22±16	19±13
Volume of particles > 40 μm, mℓ/m³	0.03±0.02	0.20±0.10	0.14±0.12
Number of particles > 40 μm; Number x 10³/m³	30±42	332±410	280±474

TABLE 2
Characterisation of secondary effluents

$$Np = cTSS^m \qquad (4)$$

where:

Np = Micro-organisms embedded in particles (CFU/100 mℓ)

TSS = Total suspended solids content (mg/ℓ)

c, m = Empirical coefficients specific to each type of effluent

Risk assessment

WHO criteria (WHO, 2006) only set values for the faecal coliform content and suggest setting additional values for other micro-organisms depending on the local situation. Therefore, to evaluate the health risks caused by the *Salmonella* Typhi content of an effluent disinfected in accordance with WHO criteria, the infection probability resulting from exposure (P) was calculated using the beta-Poisson model (Eq. (5), Westrell et al., 2004). The assumptions used for the calculation were: 100 g portion of lettuce irrigated with treated wastewater; 10 mℓ volume of wastewater remaining in the product; a 3 log reduction during harvesting and consumption; and a 1 log reduction due to washing (Fattal et al., 2004).

$$P = 1-(1+((N/\beta))^{-\alpha} \qquad (5)$$

where:

P = Risk of infection per person per consumption of 100 g of lettuce containing 10 mℓ of treated wastewater

N = Number of bacteria at the time of consumption

α = 0.33

β = 139.9

The *Acanthamoeba* spp. risk assessment has not been evaluated due to the lack of data concerning the infective dose and dose-response relationships. This lack of information is a concern

not only for developing countries but also for developed ones, because this micro-organism has been classified as an emergent pathogen by USEPA's CCL (USEPA, 1998).

Results and discussion

Characterisation of effluents and their effect on UV efficacy

UV-light efficiency depends not only on the type of micro-organisms which require inactivating but also on the effluent's characteristics, such as transmittance, the TSS content and the particle size distribution. In particular, particles shade, reflect or absorb UV-light diminishing its effect on micro-organisms. Also, UV-light efficiency decreases in effluents with TSS contents > 30 mg/ℓ, transmittance < 60% and the presence of particles > 40 μm, according to Madge and Jensen (2006).

The AS effluent came from a suspended growth biological wastewater treatment process and for that reason had the highest TSS content (31±10 mg/ℓ) and the lowest transmittance value (55±7%), presenting the worst conditions for applying UV-light. In contrast, effluents coming from the attached growth systems, RBC and TF, presented a lower TSS content (of 9±2 and 12±1 mg/ℓ, respectively) and 20% higher transmittance values (Table 2). Although the particle size distribution observed for the 3 effluents was different (Fig. 1 and Table 2), the number of particles larger than 40 μm (a size that directly affects UV dose) represents a small percentage (0.12% on average) in all effluents. In contrast, in terms of volume, particles greater than 40 μm were markedly different for the 3 effluents: 2% of the total volume for the AS effluent (0.03 m ℓ /m³), 15% (0.2 ml/m³) for the RBC one and only 7% (0.14 mℓ/m³) in the TF one (Fig. 1).

Inactivation efficiencies for each type of micro-organism studied were similar for the 3 effluents using the UV-light dose required to fulfil the WHO criteria (30 mW·s/cm²) (Table 3), meaning that differences observed in the TSS content, the trans-
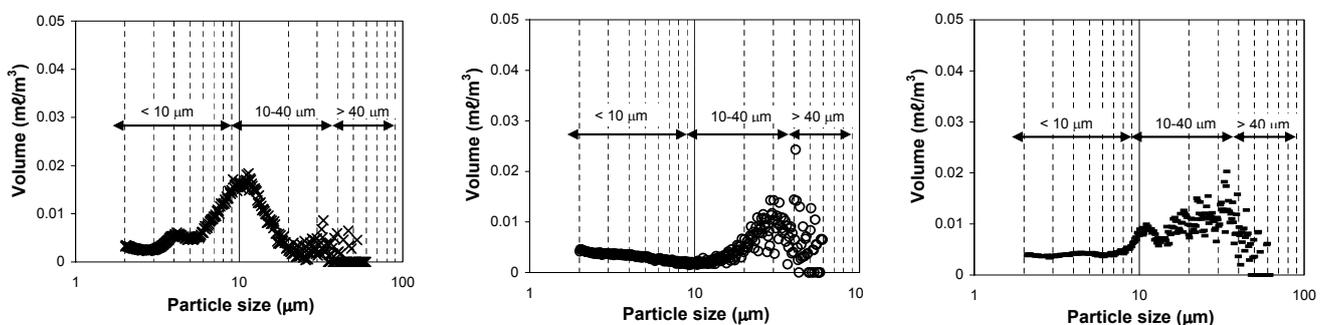

Figure 1
Particle size distribution: (a) activated sludge, (b) rotating biological contactors and (c) trickling filters

TABLE 3
Concentration of faecal coliforms, faecal enterococci and *Salmonella* Typhi for the three secondary effluents

Dose mW·s/ cm²	Log (CFU/100 mℓ)								
	Faecal coliforms			**Faecal enterococci**			***Salmonella* Typhi**		
	AS	**RBC**	**TF**	**AS**	**RBC**	**TF**	**AS**	**RBC**	**TF**
0	6.2±0.6	5.6±0.2	6.0±0.3	6.5±0.3	5.5±0.1	6.0±0.2	4.8±0.9	4.2±0.4	4.5±0.5
5	5.5±0.3	4.8±0.3	5.3±0.3	6.0±0.5	5.3±0.3	5.8±0.2	4.1±0.4	3.1±0.3	3.4±0.7
10	4.1±0.4	3.9±0.3	4.0±0.5	5.4±0.4	4.9±0.3	4.9±0.3	3.2±0.4	2.6±0.4	2.9±0.8
15	2.9±0.3	2.9±0.3	3.2±0.4	4.5±0.3	3.7±0.5	4.0±0.5	2.6±0.7	1.9±0.5	2.2±0.4
30	2.4±0.3	2.5±0.4	2.3±0.6	3.1±0.4	3.2±0.3	3.2±0.5	1.7±0.5	1.8±0.5	1.5±0.8
60	1.8±0.2	2.0±0.3	1.9±0.3	2.1±0.9	2.7±0.3	2.7±0.6	1.1±0.7	0.4±0.7	1.2±0.8
120	0.7±0.6	1.1±0.9	1.3±0.6	0.9±0.9	2.3±0.4	2.0±0.3	0.0(NA)	0.1±0.4	0.3±0.5
NA. Not applicable, AS: Activated sludge, RBC: Rotating biological contactors, TF: Trickling filters									

mittance values and the particle size distribution were not determinants in the 3 biological effluents. This was explained by the fact that they came from the same influent and had a low number of particles over 40 μm in size. Although the RBC and the TF effluent distribution curves had greater volumes of particles over 40 μm, this did not seem to affect the UV-light dose.

The UV dose-response curves (Fig. 2) show the typical inactivation phases. In the 1st phase (for doses < 15 mW·s/cm²) the inactivation of free micro-organisms not associated with particles followed a first-order reaction. The 2nd phase (tailing of the curve) was observed for UV-light doses >30 mW·s/cm².

Effects of WHO criteria UV dose on bacteria and amoebae

For all effluents, the 30 mW·s/cm² dose inactivated 3 log of faecal coliforms fulfilling the WHO criteria for agricultural irrigation and 1 000 MPN/100 mℓ (Table 3). This dose is similar to the one reported by Jacangelo et al. (2003) of 30 to 45 mW·s/cm² to inactivate 3 log of faecal coliforms in secondary effluents. Faecal enterococci, not considered in the WHO criteria or in the Mexican standard, showed a higher resistance than faecal coliforms in the 1st phase of the dose-response curve, because it was inactivated by only 2 logs. This behaviour was attributed to the ability of some micro-organisms to absorb sub-lethal irradiation doses without displaying any adverse effect measurable through the analytical techniques used to quantify their viability (Task Force on Wastewater Disinfection, 1996). Concerning *Salmonella* Typhi, it was inactivated to concentrations of below the human infective dose (10³) by applying only 15 mW·s/cm². The total log reduction achieved for this pathogen when fulfilling the WHO criteria was 3 log units. With the 30 mW·s/cm² dose *Acanthamoeba* spp. was not affected at all. A 2 log reduction was observed until a 40 mW·s/cm² dose was reached, *i.e.* two times greater than the one required to inactivate *E. coli* (Chang et al., 1985). Total inactivation of *Acanthamoeba* spp. was reached with doses of up to 173 mW·s/cm², confirming its high resistance to disinfection as frequently reported for other protozoan but also indicating that the low doses recommended for *Cryptosporidium* (2 to 3 mW·s/cm²) (Linden et al.,

2001) certainly do not apply in the case of this micro-organism. In the literature, no doses for inactivating *Acanthamoeba* spp. have been reported to compare with, but for *Acanthamoeba castellanii* a much higher dose, of 800 mW·s/cm² (Aksozek et al., 2002), has been reported. Using other disinfection methods, *Acanthamoeba* spp. has been inactivated with 3 mg ClO_2/ℓ and 7 mg O_3/ℓ, which are doses 10 and 70 times greater that those needed to inactivate bacteria, respectively (Cursons et al., 1980; Dawson and Brown, 1987). It should be noticed that the *Acanthamoeba* content used in the study was much higher (10⁴ cysts/mℓ) than the one that might be present in wastewater. Usual content of other types of protozoan, such as *Giardia Cryptosporidium* or *Entamoeba coli* and *histolytica* are lower in wastewater (10² to 10⁶, 10¹ to 10⁴, 10² cysts/ℓ respectively; Cacciò et al., 2003; Lonigro et al., 2006). So it can be assumed that the actual content of *Acanthamoeba* in wastewater could be inactivated with lower doses.

Kinetics parameter

To measure the micro-organisms' sensitivity to UV-light irradiation, the inactivation rate constant was determined using a first-
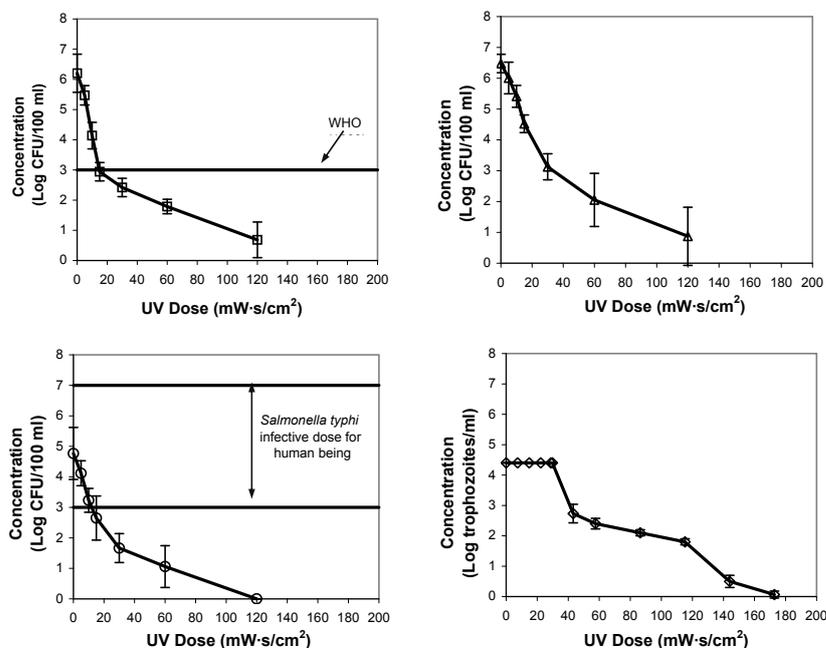
Figure 2
Dose-response curves: (□) faecal coliforms, (Δ) faecal enterococci and (O) Salmonella *Typhi and (◊)* Acanthamoeba *spp. in an activated sludge effluent*

TABLE 4				
UV inactivation rate constants of faecal coliforms, faecal enterococci, *Salmonella* Typhi and *Acanthamoeba* spp. for three secondary effluents				
	k (cm^2/mW·s)			
	Faecal coliforms	Faecal enterococci	*Salmonella* Typhi	*Acanthamoeba* spp.
Activated sludge	0.48	0.28	0.36	0.05
Rotating biological contactors	0.41	0.23	0.37	N.D.
Trickling filters	0.43	0.28	0.37	N.D.
N.D: Not determined				

order equation. Inactivation rate constants (Table 4) showed that the most sensitive micro-organisms were FC (0.48 cm^2/mW·s), although it is considered the universal indicator. The inactivation rate values obtained in this study for faecal coliforms and enterococci were similar to those reported by Moreland et al. (1998), of 0.34 and 0.28 cm^2/mW·s respectively, also using secondary effluents. In contrast, *Salmonella* Typhi inactivation rate values reported in the literature are greater (0.92 y 1.1 cm^2/mW·s) (Task Force on Wastewater Disinfection, 1996) than the value obtained in this research. This difference may be due to the greater resistance of indigenous strains compared to pure strains. This should be considered when using results obtained from the literature in ideal conditions to model local conditions.

Even though the *Salmonella* Typhi inactivation rate was slower than the one for faecal coliforms, the use of these bacteria as an indicator of the former is feasible because *Salmonella* Typhi is always present in lower concentrations in wastewater (usually 2 to 6 log less) which is not the case with faecal enterococci which are present in similar densities to faecal coliforms (10^4 to 10^7 and 10^6 to 10^8, respectively) (Tchobanoglous et al., 2003).

Acanthamoeba spp., as expected, was the most resistant micro-organism in this study, with an inactivation constant rate of 0.05 cm^2/mW·s. Its resistance is due to its cyst wall composed of a phosphoprotein layer (external) and a cellulose layer (internal) that acts as an extracellular barrier (Marciano-Cabral and Cabral, 2003). This chemically and structurally complex cell wall gives the structure opacity, protecting its genetic material from UV-light (Maya et al., 2003). *Acanthamoeba* spp. low inactivation suggests again the need to use additional microbial indicators when wastewater containing different types of pathogens, such as protozoan, are involved.

Since cysts are 15 times more resistant than vegetative bacteria, faecal coliforms may not serve as reliable quantitative models for disinfecting cysts, according to Chang et al. (1985). Thus it would be convenient not only to perform disinfection tests under laboratory conditions to define the sensitivity of different pathogens and to determine the doses needed, but also to monitor full-scale processes using indicators at adequate concentrations. With respect to bacteria, results showed the limited application of faecal coliforms as indicators and suggested the usefulness of replacing them in some justified cases such as faecal pollution indicators by faecal enterococci. The analytical procedures used to determine them are similar but the costs differ (21 USD and 32 USD for faecal coliform and faecal enterococci, respectively, USEPA, 2001) for the former being lower.

Reactivation

Faecal coliform content increased by 1.4 logs in sunlight-exposed samples treated with 30 mW·s/cm^2, exceeding criteria and standard limits for agricultural reuse in 120 min. Observed photoreactivation capacity for *Salmonella* Typhi was less than that observed for faecal coliforms. Even though they increased by a maximum of 1.9 logs with the 30 mW·s/cm^2 dose, the content did not reach the infective dose (10^3). Photoreactivation was not observed for any of the bacteria followed when doses greater than 60 mW·s/cm^2 were applied (Fig. 3). Similar results have been reported by Lindenauer and Darby (1994).

When samples were kept in the dark, a slight reactivation was observed only for faecal coliforms. Dark repair mechanism (a multi-enzymatic mechanism termed excision repair) for both faecal coliforms and *Salmonella* Typhi, was not effective even for low UV doses. The maximum growth observed in these conditions was around 0.3 logs for all the doses applied.

The reduction dose factor for faecal coliforms calculated using Eq. (3) was 0.5, meaning that the recommended dose for disinfection and avoiding reactivation needs to be at least 60 mW·s/cm^2. However, it must be borne in mind that photoreactivation rates determined in the laboratory very often represent maximum reactivation rates since other variables such as energy, death, predation and temperature present in field conditions are not involved. Thus, in field conditions photoreactivation will be less frequent (Whitby and Palmateer, 1993).
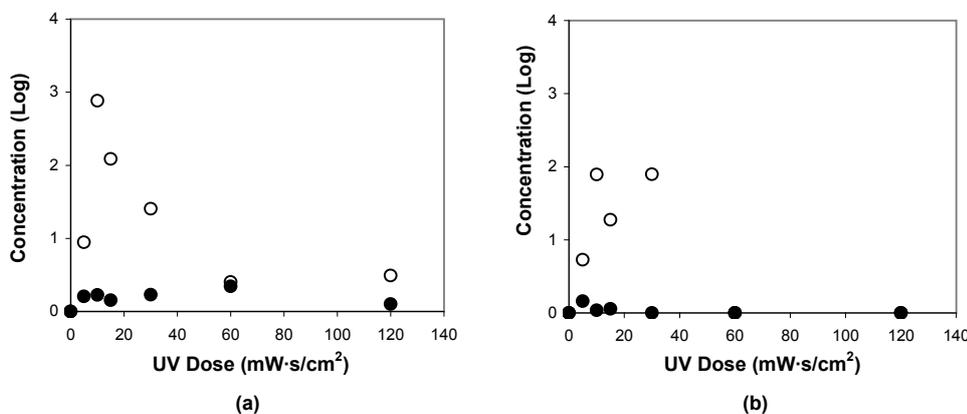
Figure 3
Concentration of (a) faecal coliforms and (b) Salmonella Typhi in treated samples exposed to sunlight (○) and dark conditions (●)

Effect of embedded micro-organisms on inactivation efficiency

Samples prepared adding TSS to the AS effluent displayed similar faecal coliform concentrations (around 7 ±0.3 log) even though the TSS content varied from 5 to 100 mg/ℓ, due to the analytical technique used to measure bacteria which cannot differentiate among bacteria embedded in flocs. The UV-light dose-response curves for the different TSS contents (Fig. 4) showed the typical behaviour, with first-order inactivation and tailing regions. All the dose-response curves obtained had similar slopes (0.217 ±0.04) indicating that the inactivation of free micro-organisms was carried out independently of the SS content. All said, the disinfection efficiency diminished as the TSS increased in the tailing region only.

However, in the tailing region, as the SS concentration increased, the inactivation rates diminished from 0.19 to 0.03 cm²/mW·s for TSS between 15 and 100 mg TSS/ℓ. For TSS contents higher than 30 mg/ℓ, the inactivation rate for the tailing region remained constant and equal to 0.02 cm²/mW·s, even when using the higher UV-light doses (Table 5). These results support the idea that better UV-light disinfection efficiencies and consequently lower costs may be achieved in effluents with less than 30 mg TSS/ℓ. Thus, it is recommended that particles be physically removed (i.e. filtration) when higher TSS contents are involved (Loge et al., 2002).

The values found for constants c and m with a correlation coefficient of 0.8 were 0.06 and 2.42, respectively. They differ notably from those proposed by USEPA (1986) of c = 0.26 and m = 1.9 that are commonly used when no empirical information is available. Therefore it is important to determine these values in practice. At higher UV doses where most of the free bacteria have been inactivated, the residual bacteria density should be close to Np. Equation (6) is based on values obtained from Eq.(4), to evaluate the minimum faecal coliform content that may be achieved with a UV-light process at different TSS content.

$$N_p = 0.06 \, TSS^{\,2.42} \tag{6}$$

Infectious risk assessment

Because it was observed that after fulfilling the WHO criteria *Salmonella* Typhi was still present, the infection risk was estimated. This was performed using the final concentration for samples treated with 30 and 60 mW·s/cm² (WHO criteria and optimal UV dose determined according to results), and considering a single consumption of 100 g of irrigated lettuce containing 10 mℓ of treated effluent, a 3-log die-off between irrigation and consumption and using the β-Poisson model (Eq. (7)). Parameters used in this equation were those recommended by Rose and Gerba (1991).

$$P_I = 1 - \left(1 + \frac{N}{139.9}\right)^{-0.33} \tag{7}$$

The infection risk value for *Salmonella* Typhi turned out to be $P_I = 1.2 \times 10^{-5}$ for 30 mW·s/cm² and of $P_I = 3.1 \times 10^{-6}$ for 60 mW·s/cm², which are acceptable according to the USEPA criteria (USEPA, 1992), which establishes a limit of $P_I = 10^{-4}$. For comparison purposes, the annual infection risk caused by salmonella when consuming 100 g of lettuce irrigated with raw wastewater varies from 1.5×10^{-1} to 5×10^{-2}, meaning that 5 to 15% of the consumers are at risk of getting the disease (Fattal et al., 2004).
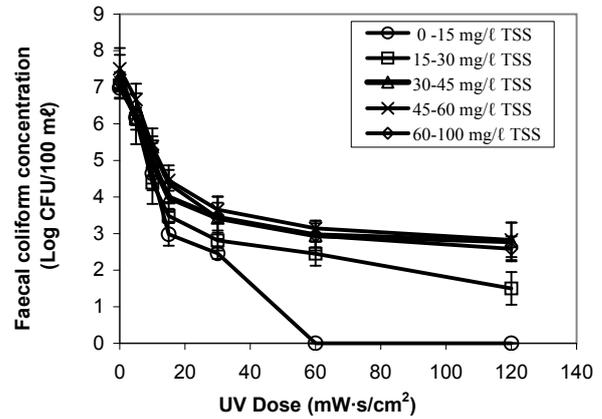

***Figure** 4*
Effect of total suspended solids concentration on faecal coliforms' dose response

TABLE 5 Faecal coliform inactivation rate constants for the tailing region		
Total suspended solids concentration (mg/ℓ)	k (cm²/mW·s)	R²
0-15	0.19	1.00
15-30	0.03	1.00
30-45	0.02	0.80
45-60	0.02	0.90
60-100	0.02	0.93

Conclusions

A dose of 30 mW·s/cm² is enough to fulfil WHO criteria and to inactivate 3 log *Salmonella* Typhi and faecal enterococci in the three different effluents (AS, RBC and TF) coming from the same influent, even though the TSS content and the particle size distribution were different. This was explained by the amount of particles > 40 μm in the 3 effluents being low. For any of the effluents, the disinfection efficiency is affected by the presence of micro-organisms embedded in particles. No *Acanthamoeba* inactivation was observed at 30 mW·s/cm²; however, a 2.5 log reduction of *Acanthamoeba* as well as an average of 4 log for bacteria (faecal coliform, faecal enterococci and *Salmonella* Typhi) is reached when applying the recommended UV-light dose to fulfil the WHO criteria and to avoid photoreactivation (60 mW·s/cm²). However, if it is necessary to fully inactivate it, this dose would need to be tripled and UV disinfection may turn into an economically nonviable process. Since *Acanthamoeba* trophozoites and cysts are relatively large, physical treatment processes such as flocculation, sedimentation and filtration can be effective in their removal.

The mean inactivation rate constants calculated in the linear region of the disinfection curves for the 3 effluents (0.4 cm²/mW·s for faecal coliforms, 0.26 cm²/mW·s for faecal enterococci, 0.37 cm²/mW·s for *Salmonella* Typhi and 0.05 cm²/mW·s for *Acanthamoeba* spp.) showed that the most sensitive micro-organisms were faecal coliforms, although these are generally considered the usual indicator. Considering the resistance of faecal enterococci, their use as an indicator besides faecal coliforms, should be evaluated. Also, since there is minimal to no correlation between bacterial indicators and protozoa, the use of other types of indicators, particularly in developing countries, should also be considered.

The health risks produced by effluents disinfected with 30 mW·s/cm^2 and 60 mW·s/cm^2 are between 0.0003 and 0.001% for *Salmonella* Typhi, which is acceptable. However, even though the risk assessment for *Acanthamoeba* spp. could not be quantified because of a lack of information, the probability of infection still exists. Even though it is rarely possible, and generally quite unnecessary, to use sterile or pathogen-free water for irrigation, it is suggested that biological parameters other than conventional indicators be considered during experimental studies to perform the design of UV-light facilities in order to safely reuse wastewater for agriculture in developing countries. As a result, public health may be protected without increasing routine monitoring costs, particularly in several regions from the developing world where wastewater is reused.

Acknowledgements

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