Inactivation of *Salmonella* spp. from secondary and tertiary effluents by UV irradiation

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Abstract The aim of this study was to verify the efficiency of UV irradiation in the inactivation of *Salmonella* spp. in treated wastewater with different levels of turbidity and exposed to increasing doses of UV irradiation. Experiments were carried out in a batch reactor and in a real scale reactor. Salmonellae obtained from clinical samples were seeded into autoclaved wastewater collected from a wastewater treatment plant (WWTP) comprising an association of a UASB reactor followed by three submerged aerated biofilters (BAF) and one tertiary filter. The results showed that salmonellae were not inactivated in effluents from the UASB reactor indicating that the presence of suspended solids was an important obstacle to UV penetration in bacteria. However, UV irradiation was efficient in inactivating *Salmonella* of effluents from aerated secondary and tertiary biofilm reactors.

Keywords Disinfection; *Salmonella* spp.; UV irradiation; wastewater

Introduction

Despite technological advances in water and wastewater treatments, contaminated water is still an important vehicle of transmission for enteric pathogens of humans and animals. Untreated waters are normally discharged into surface waters, especially in undeveloped countries, and gastroenteritis is a major contributor to morbidity and mortality of children under 5 years old in these regions.

The direct search for pathogenic microorganisms from water and wastewater samples presents several problems, such as their low numbers and their intermittent presence. These disadvantages have led to the use of other microorganisms (indicators) which are easier to analyse. Total and faecal coliforms (TC, FC) and faecal streptococci (FS) are the classical indicators; however some studies have reported the detection of several pathogenic microorganisms in absence or at low levels of these indicators (Moriñigo *et al*., 1990a). Among these pathogens are *Salmonella* spp., enteric bacteria responsible for elevated numbers of gastrointestinal infections. Salmonellae have been isolated from fresh, waste and marine waters (Baudart *et al*., 2000a,b; Catalao Dionisio *et al*., 2000) and can be an alternative indicator of the microbiological wastewater quality. Although the majority of wastewater treatment plants are able to product effluents with physicochemical characteristics within international norms, the inactivation and elimination of pathogenic microorganisms, such as salmonellae, is more difficult to attain. Alternative methods, such as UV irradiation, have been proposed for disinfection of water and wastewater after conventional treatment. This study aimed to verify the efficiency of UV irradiation in the inactivation of *Salmonella* spp. in treated wastewater with different levels of turbidity and exposed to increasing doses of UV irradiation.

Materials and methods

Pilot plant description and performance

The study took place in a WWTP receiving domestic sewage (planned to serve 1,000 inhabitants) and constituted a UASB reactor (35 m^3) and four submerged aerated biofilters

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(12 m^3). The average values obtained from the monitoring of the plant indicated COD = 445 mg/L and TSS = 168 mg/L in the raw sewage. The UASB reactor achieved 62% efficiency in the removal of organic matter (COD = 191 mg/L and TSS = 62 mg/L from primary effluent), decreasing the organic matter affluent in the aerobic reactor and minimising costs due to energy consumption with aeration. The final effluent parameters achieved TSS <30 mg/L and COD <90 mg/L with removal efficiency of 78% of the carbonaceous substrate. As a result, the reactors may have smaller volumes maintaining the same effluent quality. The bottom sludge was 5% total solids (θ = 11 h). Although the returned sludge from the biofilters had VS concentration of 80%, the combined sludge kept VS concentration at a 59% level. Sludge production in the biofilters was estimated at 1.44 kg TSS/d while the UASB reactor gave a sludge production rate of 2.29 kg TSS/d The biogas was produced at 190 L$_{biogas}$/kgCOD$_{rem}$ equivalent to 7 L/inhabitant.day. Considering that 70% of the biogas was formed by methane, and allowing 30% energy utilisation efficiency, methane production of 1.3 kgCH$_4$/d generated an energy amount of 0.23 kW/d (5W/inhabitant.day).

Irradiation experiments

Laboratory scale. Samples from raw sewage and effluents from primary treatment (UASB), secondary treatment (BF2) and tertiary treatment were used in the irradiation experiments. Control samples, non-irradiated, were also performed. *Salmonella* spp. obtained from clinical isolates were seeded (about 6×10^5) in beakers with 120 mL autoclaved effluents and irradiated. The irradiation experiments were performed in a batch reactor that consisted of a germicidal lamp suspended 40 cm above the exposure area. Collimated radiation was measured with a radiometer (MOD.UVC 254-COD.2056- Lutron Instruments) using a detector for incident intensity at 254 nm wavelength. UV doses of 10, 20, 30, 50, 75 and 100 mWsec/cm^2 were applied to the samples. Wastewater was stirred during irradiation using stir bars.

Real scale. Experiments were done in a compact UV reactor of submerged lamps. Low pressure mercury UV lamps (30W; ×26), spaced 10 cm apart, were transversally positioned to the flow of liquid. The reactor continually received the effluent of tertiary treatment.

Salmonella assay

After irradiation with different UV doses, samples were immediately cultured in selective selenite medium (with novobiocine) for 24 h followed by culture on XLD or Brillant Green agar with the MPN being determined after 24 h (CETESB, 1993). For real scale experiments, 1×10^6 *Salmonella* were seeded into a 310 L box with tertiary effluent from the WWTP. The artificially contaminated effluent was allowed to flow for 10min in the reactor and samples were collected at the beginning, the middle and at the end of the reactor. To avoid any contamination of the water bodies, 5% chlorine was added to eliminate microorganisms not inactivated by UV irradiation during the test.

Physicochemical parameters

Chemical analyses were performed according to *Standard Methods* (APHA, 1995). The parameters analysed were absorbance at 254 nm (Ultrospec 1000 UV spectrophotometer, Amersham, using a quartz cuvette with 1 cm path length and deionised water as a blank), turbidity (NTU; Lamotte – mod 2020) and total suspended solids (TSS). Tertiary effluents were filtered with 0.8 µm membranes to achieve different levels of turbidity.

Results and discussion

Laboratory scale

The results showed that the number of viable *Salmonella* in treated wastewater effluents could be considerably reduced after UV disinfection. In raw wastewater and UASB effluent from the WWTP the inactivation of *Salmonella* was not significant, indicating that the quantity of suspended solids was an important obstacle to UV penetration in bacteria. However, samples from effluents of secondary and tertiary treatment have lower quantities of suspended solids and presented better inactivation of *Salmonella* after irradiation. The inactivation kinetics of *Salmonella* seeded in secondary and tertiary effluents after UV irradiation in a batch reactor are presented in Figures 1–3. The physical-chemical parameters of the effluents are indicated.

These results showed that in the secondary effluent the inactivation was more effective with doses of UV >50 mWsec/cm^2 and resulted in a 2-log reduction of bacteria. The effluents from the tertiary reactor presented a similar behaviour of reduction of bacteria after UV irradiation.

To verify the influence of turbidity on *Salmonella* inactivation by UV light, assays were conducted with tertiary effluents at different turbidity levels. These effluents were also filtered to achieve low turbidity levels. Figure 2 shows that in non-filtered tertiary effluents *Salmonella* was sensitive to UV doses of 30 mWsec/cm^2 with a 2-log reduction being achieved. The inactivation curve showed a resistant tail after 2-log reduction that may be caused by the presence of a resistant sub-population of bacteria or by the presence of suspended solids. Such behaviour was also obtained in experiments with faecal coliforms, *E. coli* and bacteriophages indicating that they were affected in a similar way by the UV irradiation (data not presented). The results with filtered tertiary effluents showed that doses of
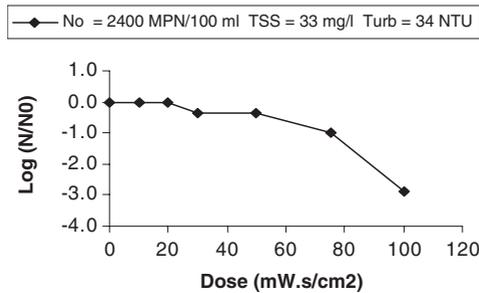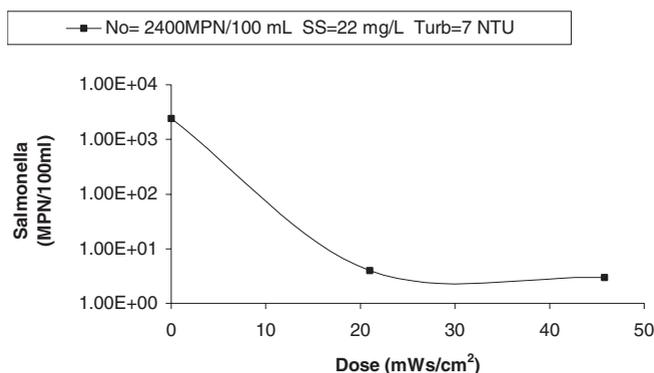
Figure 1 Disinfection of *Salmonella* with UV irradiation in the effluent of secondary reactor
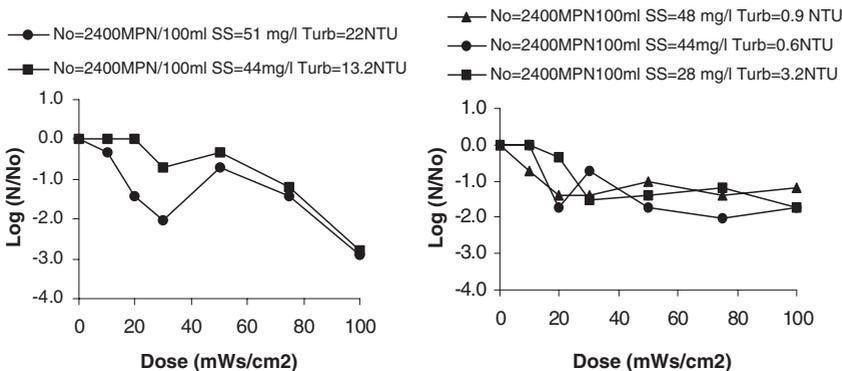
Figure 2 Disinfection of *Salmonella* with UV irradiation in effluents from tertiary reactor with different levels of turbidity
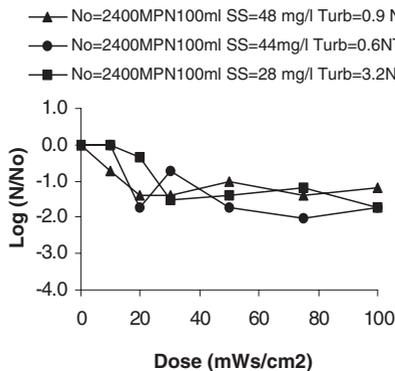
Figure 3 Disinfection of *Salmonella* with UV irradiation from tertiary effluents filtered to reach different levels of turbidity

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Figure 4 Real scale results of disinfection of *Salmonella* with UV irradiation in effluents from tertiary reactor

UV as low as 20 mWsec/cm^2 were sufficient to inactivate *Salmonella* although it resulted only in 2-log reduction of bacteria as observed for effluents with greater turbidity.

Real scale experiments

The laboratory studies allowed establishment of the conditions to conduct experiments in real scale with a UV reactor receiving continuously the effluent of the tertiary reactor. Figure 4 shows the decay curve of *Salmonella* at three different points of the reactor. The inactivation kinetics of *Salmonella* in this controlled system showed a reduction of more than 2-log between the initial point of irradiation and the end of the reactor, and a UV dose of 20 mWs/cm^2 was enough to inactivate *Salmonella* to acceptable levels for discharge. Increased doses did not have any effect on decay. In this study the hydraulic retention time was 90s which allowed bacteria to be exposed for enough time to be inactivated at low doses of UV.

Conclusion

Our results showed that the number of *Salmonella* in the effluent of a WWTP, with secondary and tertiary treatment of wastewater, could be considerably reduced by disinfection with ultraviolet irradiation. The inactivation was more efficient in effluents with low suspended solids levels as was observed with the effluent of the tertiary reactor. More than 2-log inactivation was achieved with a UV dose of 20 mWs/cm^2 in the real scale reactor.

Acknowledgements

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