Elimination of enteric indicators and pathogenic bacteria in secondary effluents and lake water by solar disinfection (SODIS)
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ABSTRACT
The reduction of enteric indicators (fecal coliforms (FC) and Enterococcus faecalis) and elimination of pathogenic bacteria (Salmonella spp. and Staphylococcus aureus) in the secondary effluents and lake water by solar disinfection (SODIS) was studied in this article. FC, E. faecalis, Salmonella spp. and S. aureus were isolated and enumerated using membrane filtration techniques after SODIS of samples inside transparent polyethylene terephthalate (PET) bottles for 1, 2, 3, 4, 5, 6, 7 and 8 h. The results show that SODIS can reduce numbers of FC, Salmonella spp. and S. aureus by more than 4 log_{10} colony forming units (CFU)/100 mL after 6 h. However, regrowth of these bacteria was observed after the incubation of the treated samples at 37 °C for 24 h, whereas SODIS for 8 h would eliminate pathogenic bacteria and no regrowth would be observed in these samples as determined by an absence and presence technique using enrichment medium. E. faecalis was not eliminated in the secondary effluents and lake water by SODIS, but this bacterium was reduced to less than detection limits (1 CFU/100 mL) when the treated secondary effluent samples were stored for 16 days at room temperature. The elimination of pathogenic bacteria and reduction of enteric indicators resulted in undetectable levels using SODIS for secondary effluents and lake water.

Key words | enteric indicator, pathogenic bacteria, regrowth, secondary effluent, solar disinfection, storage period

INTRODUCTION
The rapid growth of communities and cities during the recent two decades has led to increased amounts of human wastes (domestic, industrial and hospital waste) and the need for these to be managed safely. In most poor communities, the most important pollutant that represents primary concern is human excreta (domestic sewage). It has been reported by the World Health Organization (WHO) that 3.2 million children under the age of five die each year in the developing world due to diarrheal diseases, mainly as a result of poor sanitation and contaminated drinking water (WHO 1992).

Primary and secondary processes of domestic sewage reduce pathogenic bacteria by 95–99%. However, secondary effluents still contain high concentrations of these pathogens in effluents even after sewage treatment (Koivunen & Heinen-Tanski 2005). Thus, secondary effluents require various kinds of further treatment to reduce the density of pathogenic bacteria to ensure that favorable sanitary effluent quality is achieved. Disinfection emphasizes the reduction of pathogens to less than detectable limits and meets the requirements of environmental or microbiological requirements for effluent reuse.

Many technologies are used for disinfection of secondary effluents and reduction of microorganisms, such as chlorination (Coronel-Olivares et al. 2011), filtration
(Dungeni et al. 2010), and ozone (Tripathi et al. 2011). However, microbial resistance to chemical materials among different types of microorganisms (Xu et al. 2007; Dungeni et al. 2010) in addition to occurrence of bacterial regrowth after the treatment by these techniques has been reported (Guo et al. 2011).

The use of solar disinfection (SODIS) based technologies is a promising approach to the disinfection of water because of the high availability of solar radiation as well as the low cost and sustainable nature of these water treatment methods (Gomez-Couso et al. 2009). According to WHO (2002), SODIS is a promising new technology that uses clear plastic bottles to purify water. Bottles are filled with clear water and left in the sun for several hours. The combination of ultraviolet radiation and high temperature is able to destroy most pathogens. SODIS will not improve the chemical quality of water, but it can provide an inexpensive, easy way to improve microbiological quality.

Meierhofer & Wegelin (2002) recommended that the transparent polyethylene terephthalate (PET) bottles containing untreated raw water should be exposed to direct sunlight for at least 6 h. Bacteria, viruses, Giardia and Cryptosporidium cysts, and parasite eggs can all be effectively inactivated through the combination of ultraviolet radiation and elevated water temperature. Large field tests of SODIS are currently being conducted in a number of countries in South America, Africa, and Asia (Acra et al. 1989).

The aim of this article was to study the effectiveness of SODIS in the reduction of enteric indicators (fecal coliforms and Enterococcus faecalis) and the elimination of pathogenic bacteria (Salmonella spp. and Staphylococcus aureus) in lake water and secondary effluents. The concentrations of fecal coliforms (FC), E. faecalis, Salmonella spp. and S. aureus were determined in untreated secondary effluents and lake water as well as after treatment periods (1, 2, 3, 4, 5, 6, 7 and 8 h). The ability of residual bacteria in the secondary effluents treated for 1, 2, 3 and 4 h to survive during storage periods (2, 4, 8 and 16 days) was tested. The efficiency of SODIS in the elimination of bacteria was evaluated by studying the occurrence of regrowth of enteric indicators and pathogenic bacteria in treated secondary effluents during the incubation time of treated samples for 24, 48, 72 and 96 h at optimal conditions of bacteria (37 °C) and by using enrichment media.

**MATERIALS AND METHODS**

Isolation and enumeration of bacteria from the samples

Secondary effluents and lake water samples were collected from a sewage treatment plant (STP) that treats hospital domestic sewage and a lake containing rain water, respectively. The treatment process in the STP includes primary sedimentation and oxidation pond. The samples were aseptically collected in sterile bottles (Duran, Germany) and transferred to the laboratory and stored in the refrigerator (the maximum storage time, overnight) for bacterial analysis.

Samples were thoroughly mixed at least 25 times to distribute the bacteria uniformly prior to analysis. Fecal coliforms, Salmonella spp., S. aureus and E. faecalis were enumerated using membrane filtration techniques on appropriate selective media according to internationally accepted techniques and principles (Benson 2004; USEPA 2006a, b) with some modification.

Thirty milliliters of each sample was suspended in 270 mL of sterilized distilled water. Serial dilutions (10^{-1}–10^{-4}) were prepared, and then 10 mL of each dilution was filtered through a Millipore cellulose membrane (0.45 μm) (Microsart, Sun Sri-Germany). The filters were then placed into endo agar media (M029) to count FC; xylose lysine deoxycholate agar (XLD, M013) for Salmonella spp.; mannitol salt agar (MSA, M-118) for S. aureus; and bile esculin iron agar (BEIA, M972) for E. faecalis (all media were supplied by HiMedia Laboratories, Pvt. Ltd, India).

Media were sterilized by autoclave (Model ES-315, Tomy-Japan) at 121 °C for 15 min, except for XLD, which was sterilized by boiling. Experiments were carried out inside a horizontal laminar flow cabinet (Model AHC-4A1-ESCO). Plates were incubated (Memert incubator-Germany) for 24–48 h at 37 °C for Salmonella spp. and S. aureus and at 44.5 °C for FC and E. faecalis.

The colony numbers of the bacteria were determined by simply counting based on the appearance of the morphology and color of the colonies by using a colony counter (Funke
Gerber-Germany) calculated according to the method described by USEPA (2006a, b) and reported in terms of log10 colony forming units (CFU) of the bacteria per 100 mL of the sample. The limit of detection is 1 CFU/100 mL of sample (equivalent to zero log10 CFU/100 mL) (Clesceri et al. 1998).

A greenish metallic sheen or pink color indicated FC on the endo agar. *Salmonella* spp. were red in color with black centers. The colonies of *E. faecalis* grown on BEIA had brown centers, confirmed by a catalase test. Colonies of *S. aureus* on MSA with a gold color were also counted and confirmed by catalase and coagulase test.

*Salmonella* spp., *S. aureus* and *E. faecalis* were identified based on morphological and cultural characteristics and biochemical tests by using API 20 Strep (Ref 07226 B) and API 20NE (Ref 07224 B) (BioMerieux, SA-France) according to Kenner & Clark (1974), Hart & Shears (2001) and Brenner et al. (2005).

**pH and temperature measurements**

The pH and temperature of secondary effluents and lake water samples were measured directly using a pH meter (Sens ion 3-Model 51935- Electrode w/tem-5 pin-HACH-USA).

**Solar disinfection of secondary effluents and lake water**

Four hundred milliliters of secondary effluent and lake water samples was placed into the PET bottle (600 mL) and exposed to sunlight during the period 11.00 am–3.00 pm according to studied times, which were 1, 2, 3, and 4 h (first day) and 5, 6, 7 and 8 h (second day). The pH and temperature of water flowing in and out of the SODIS unit were monitored for all runs. Samples were collected from the SODIS and concentration of FC, *Salmonella* spp., *S. aureus* and *E. faecalis* bacteria were counted following incubation as mentioned previously. After 8 h of SODIS the pathogenic bacteria were counted or enriched by using Brain Heart Infusion medium (BHI medium) to study the presence or absence of *Salmonella* spp., *S. aureus*, *E. faecalis* and FC, respectively. Ten milliliters of each treated sample was transferred to BHI broth (enrichment media), incubated for 6 h and then 1 mL of the enrichment media was cultured on XLD, MSA, BEIA and Endo agar medium to confirm the presence or absence of *Salmonella* spp., *S. aureus*, *E. faecalis* and FC, respectively.

**Storage of treated secondary effluent samples after solar disinfection**

Secondary effluent samples that were treated by solar radiation for periods of 1, 2, 3 and 4 h were stored in closed bottles to accomplish the inactivation of fecal indicators and elimination of pathogenic bacteria. *Salmonella* spp., *S. aureus*, *E. faecalis* and FC were counted or enriched after 2, 4, 8 and 16 days of storage at room temperature.

**Regrowth of pathogenic bacteria in treated secondary effluents after solar disinfection**

All samples treated by solar radiation for periods 5, 6, 7 and 8 h were incubated at 37 °C for 24, 48, 72 and 96 h to study the occurrence of regrowth of enteric indicators and pathogenic bacteria. After incubation time enteric indicators and pathogenic bacteria were counted or enriched as described above.

**RESULTS AND DISCUSSION**

**Reduction of enteric indicators and elimination of pathogens by SODIS**

Reduction of enteric indicators and elimination of pathogenic bacteria in the secondary effluents and lake water samples by SODIS were studied in this research. The results showed that during the SODIS process the temperature in the secondary effluents and lake water ranged from 43 to 46 °C during the SODIS process compared to the control, 24–27 °C (secondary effluents without SODIS) (Figure 1). FC numbers decreased with increasing SODIS time (1–8 h) in both secondary effluents and lake water, while they remained at constant levels in the control samples (5.33 log10 CFU/100 mL). The inactivation rate of FC in lake water was more than that in the secondary effluents. SODIS of lake water and secondary effluents reduced FC by more than 4 log10 CFU/100 mL after 6 h, whereas after 7 and 8 h they were undetectable (<1 CFU/100 mL) (Figure 2).
A 3-log reduction in *Escherichia coli* concentration by solar irradiation of contaminated water in a batch system at about 5 h has been reported (Wegelin et al. 1994). Caslake et al. (2004) have reported that SODIS eradicated more than 4 log (99.99%) of total coliforms within 30 min in midday summer sunlight.

The investigations revealed that at 45°C water temperature, a synergistic effect of UV-A radiation and temperature decreases the required exposure time to attain a 4-log inactivation of fecal coliforms by 75% (Berney et al. 2006a).

The inactivation of *E. faecalis* in the secondary effluents and lake water differed from that of FC, *Salmonella* spp. and *S. aureus*. *E. faecalis* remained in concentrations more than other bacteria at all times of treatment in the SODIS process. The maximum reduction of *E. faecalis* was recorded after 8 h, 4.49 and 4.5 log_{10} CFU/100 mL of the secondary effluents and lake water respectively (Figure 2). The concentrations of *E. faecalis* in the control samples were on average 5.66 log_{10} CFU/100 mL.

One day of SODIS treatment of hygienically unsafe drinking water in PET bottles leads to a significant increase in microbiological water quality (Bosshard et al. 2009). However, on cloudy days, an exposure period of 48 h or more may be required to achieve inactivation of indicator bacteria to below detectable levels (Oates et al. 2003) and sometimes even 2 days are not sufficient (Parsons 2002). Gomes et al. (2009) found that inactivation of *E. faecalis* by solar radiation was slower than *E. coli* and reported that this may be due to the cell wall composition of the Gram-positive and Gram-negative bacteria, respectively.

The inactivation of *Salmonella* spp. in the secondary effluents was significantly different from that of the lake water at all times of SODIS (Figure 2). Elimination of *Salmonella* spp. was recorded after 6 h in the lake water but after 8 h in the secondary effluents. In comparison with FC, *Salmonella* spp. showed some resistance to SODIS treatment for secondary effluents between 1 and 4 h, which was reduced by log 1.59 and 2.98, respectively, whereas FC were greatly reduced by log 2.40 and 3.49, respectively. In contrast FC were more resistant than *Salmonella* spp. for the lake water between 1 and 4 h SODIS treatment, where they were reduced by log 1.09 and 2.30, respectively, compared to log 1.43 and 2.43, respectively, for *Salmonella* spp. USEPA (2003) demonstrated that the *Salmonella* spp. are bacteria of great concern as well as
being good representatives of reduction of other bacterial pathogens because they are typically present in higher densities than other bacterial pathogens.

As in the results of *Salmonella* spp., the inactivation of *S. aureus* in the secondary effluents and lake water was significantly different. *S. aureus* was eliminated from lake water and secondary effluents after 7 and 8 h of SODIS, respectively (Figure 2). *S. aureus* was less resistant to UV disinfection of gray water effluents than FC (Gilboa & Friedler 2008).

**Survival of enteric indicators and pathogenic bacteria during the storage periods**

The survival of fecal indicators and pathogenic bacteria in the treated secondary effluents stored for 2, 4, 8 and 16 days was tested. Storage of treated samples for 16 days significantly affected FC and *E. faecalis*. Both bacteria decreased to less than detection limits (1 CFU/100 mL) in samples treated for 4 h and stored for 16 days at room temperature (Figure 3).

In contrast, the storage period of samples treated by SODIS (4 h) did not significantly affect the inactivation of *Salmonella* spp. and *S. aureus* (Figure 3), where numbers of both pathogenic bacteria were still around $1.5 \log_{10}$ CFU/100 mL until after storage of 16 days.

The storage of secondary effluents has become the option selected in many countries because of the advantages they perform compared to other treatment alternatives (Barbagallo et al. 2003). These results are in agreement with Deportes *et al.* (1998), who reported that during storage the fecal indicators and pathogenic microorganisms remained either undetectable or at low levels. The decreases in the numbers of FC and *E. faecalis* during the storage period may be related to a decrease in pH values, which were recorded to be $<\text{pH} 4$ after 16 days of storage, while at the beginning of storage this was 6.8. The decreases in pH value may be due to anaerobic processes at the hydrolysis stage which leads to the production of acids, such as lactic acid, in anaerobic conditions.

In the preliminary study, the FC and *E. faecalis* density in the secondary effluents without SODIS dropped to below $2 \log_{10}$ CFU/100 mL after being stored for 4 weeks at room temperature. However, the concentration of *Salmonella* spp. remained high. Bacteria do not require a host cell for replication, thus they might multiply in the environment under favorable conditions (Ceustermans *et al.* 2007) such as pH, temperature and osmotic pressure. *Salmonella* are resistant microorganisms that readily adapt to extreme environmental conditions and have the ability to survive under hostile environmental conditions (Droffner & Brinton 1995). These characteristics make them the indicator of choice for monitoring the effectiveness of biosolids pathogen reduction (USEPA 1995).
Regrowth of enteric bacteria and pathogens in treated secondary effluents

The numbers of pathogenic bacteria after SODIS for 4 h were high, so the samples were subjected to the storage periods to accomplish the reduction. However, after 5 h and more the concentration of pathogenic bacteria decreased significantly, so the samples were subjected to a regrowth study. In the treatment of wastewater the major problem is the occurrence of regrowth, because some bacteria enter the dormant state phase, which means these bacteria are viable but non-culturable at the unaffordable conditions, especially in the case of secondary effluents due to more organic materials being contained.

The occurrence of regrowth of fecal indicators and pathogenic bacteria has been reported by many authors. The regrowth and repair potential of damage caused by UV irradiation of E. coli, FC strains and B. subtilis in the effluents have been observed by Guo et al. (2011). E. coli was detected after ozonation (Luczkiewicz et al. 2011). Fecal indicators are capable of regrowth in chlorinated secondary effluent (Hillel et al. 1973). Therefore, the effectiveness of any treatment method in the disinfection of secondary effluents depends on the occurrence of regrowth after the treatment process. The effectiveness of SODIS has been proven by cultivation based techniques with E. coli and some pathogenic organisms (Berney et al. 2006b). In this work the effectiveness of SODIS was evaluated by studying the occurrence of regrowth of bacteria in treated samples.

The regrowth of enteric indicators and pathogenic bacteria in secondary effluents treated by SODIS for the exposure periods of 5, 6, 7 and 8 h was studied followed by incubation of treated samples at optimal conditions (37°C) for 24, 48, 72 and 96 h incubation periods. The results found that the numbers of FC in samples treated for 5 and 6 h increased significantly from 1.69 to 3.12 and about 1 to 2 log_{10} CFU/100 mL, respectively, after the incubation period of 96 h at 37°C, while there was no regrowth of FC after the SODIS treatment for samples treated at 8 h exposure times (Figure 4). Gomes et al. (2009) reported that no bacterial regrowth was observed in dark conditions during 24 h after illumination of E. coli suspension by SODIS.

The amount of regrowth in E. faecalis was more than that of FC. Numbers of E. faecalis increased significantly in all samples treated and incubated at 37°C to different times. The maximum regrowth of E. faecalis was observed in samples treated by solar irradiation for 5 h, where 3.6 log_{10} CFU/100 mL was observed (Figure 4). Fecal enterococci are a more reliable indicator than fecal coliforms for the detection of microbial pollution because fecal enterococci are more resistant in the environment than fecal coliforms (Harvey & Garabedian 1991; Celico et al. 2004).

The regrowth of Salmonella spp. in samples treated for periods of 5 and 6 h increased with an increase of incubation time at 37°C. The maximum regrowth was 3.06 log_{10} CFU/100 mL and was observed in samples treated for a period of 5 h and incubated for 96 h (Figure 4).
However, no regrowth was observed of *Salmonella* spp. in samples treated by SODIS for the period of 8 h, and these results were confirmed by enrichment medium as described above under Materials and methods. Bosshard *et al.* (2009) suggest that a relatively small light dose is enough to irreversibly damage the cells of *Salmonella typhimurium* and that storage of bottles after irradiation does not allow regrowth of inactivated bacterial cells.

Regrowth of *S. aureus* in treated samples by solar radiation for 5 and 6 h was observed in up to 96 h incubation periods. The maximum regrowth of *S. aureus* was noted at 96 h incubation of samples at 37 °C, where 2.6 log10 CFU/100 mL was recorded (Figure 4). However, *S. aureus* was undetectable (<1 CFU/100 mL) in samples treated for 8 h of SODIS treatment. The absence of *S. aureus* was confirmed by using enrichment medium. Similar results were noted for *S. aureus* in gray water effluents treated by UV disinfection (Gilboa & Friedler 2008).

**CONCLUSIONS**

It can be concluded that SODIS of secondary effluents and lake water for 8 h is efficient at eliminating pathogenic bacteria and reduces enteric indicators to less than detection limits (<1 CFU/100 mL).

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