Evaluation of different disinfection techniques for the treatment of bacteria in suspension and biofilm.

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ABSTRACT

In this study, we investigated the use of ultrasound as an alternative for more traditional techniques like chlorination and UV irradiation for the disinfection of water. A pilot plant designed to compare traditional disinfection techniques with ultrasound was used to asses the potential benefits of using ultrasound in a closed loop configuration. The pilot plant consists out of four water containers with pumps to adjust the water flow, a piping system, a disinfection unit (chlorination, ultrasound or UV irradiation) and four biofilm monitors with 18 polycarbonate plates on which a biofilm can be formed. The disinfection efficiency of the different techniques is evaluated and the operational costs for the different techniques is assessed. Quantification of the number of bacteria is carried out with traditional plate counts on Brain Heart Infusion Agar. Water (100 l) inoculated with a bacterial suspension (10^5 CFU/ml) was treated in closed loop during 180 minutes with chlorination (0.2 mg/l free chlorine), UV irradiation (0.34 kWh) and ultrasound (0.85 kWh). Based on disinfection efficiency and operational cost the use of chlorination is, in comparison with UV irradiation and ultrasound, the most beneficial method to treat bacteria in suspension. In the treatment of larger volumes of contaminated water ultrasonic technology can not compare with the traditional techniques, partially due to a high energy demand. In the treatment of biofilm, the results of UV irradiation and ultrasound are comparable with a slightly higher energy consumption for the ultrasonic treatment.

INTRODUCTION

In several industrial processes, large flows of cooling water are utilized for the cooling of fluids and gases by means of heat exchangers. Due to environmental legislation, the heated water can not be disposed directly into surface or sewage water, but must be cooled in for instance a cooling tower. Cooling of water in such a cooling tower is achieved by heat exchange with the surrounding air, which makes it prone to contamination with micro-organisms. In such a system, microbial growth is facilitated due to the presence of nutrients, favourable temperatures, high residence time, high ratio of surface area to volume, etc (Cloete et al., 1992). This makes cooling water systems sensitive to fouling (biofilm) through the accumulation of such microorganisms on piping and equipment surfaces. The major economic impact of biofilms on cooling water systems is through energy losses due to increased fluid frictional resistance and reduced

heat transfer at power plant condensers and process heat exchangers. Biofilms accumulating on the surfaces of heat exchange tubes significantly reduce the heat transfer rate because the thermal conductivity of biofilms is significantly less, compared to the metal heat transfer surface materials (Ludensky, 2003).

The objective of biocide treatment of cooling water systems is to keep heat exchangers relatively clean from any biofouling. A variety of physical and chemical techniques are routinely used for water disinfection including chlorination (Kim et al., 2002) and ultraviolet light (Pozos et al., 2004).

Chlorination is widely used for biocidal treatment of water due to its simplicity and effectiveness. Chlorination inactivates microorganisms through a series of effects including disturbance of cell permeability, and damage to bacterial nucleic acids and enzymes. All these mechanisms play a role in the disinfection of micro-organisms but which mechanism is predominating depends on the type of microorganism and the characteristics of the treated water (Mason and Tiehm, 2001). The effectiveness of the chlorination process can be improved by increasing its concentration. This may, however, exacerbate some of the problems associated with chlorination which include: (i) reaction with dissolved chemicals to produce toxic by-products; (ii) the build-up of resistance in micro-organisms to chlorination; (iii) discoloration and the production of unpleasant flavours; and (iv) ineffective inactivation of microorganisms which reside within bacterial agglomerates. There are two ways in which such problems can be overcome either by finding an alternative biocide that does not generate toxic by-products or by reducing the concentration used for chlorination while maintaining effectiveness (Duckhouse et al., 2004).

Over the years UV irradiation is grown to a well known technique for the disinfection of water. The most widely used UV lamps have a peak emission at 254 nm and are used to kill or inactivate micro-organisms and prevent bacterial growth. The UV irradiation shall penetrate the bacterial cell membrane and will cause DNA damage which results in cell death (Kano et al., 2003). The potency of a irradiation technique like UV treatment is limited in highly light scattering or absorbing solutions, or when microorganisms are capable of photoreactivation (self-repair) (Hua and Thompson, 1999).

It is clear from the above that the drawbacks of currently used disinfection techniques, such as chlorination and UV irradiation, necessitate the exploration for alternative disinfection techniques. Therefore, the potential of ultrasonic irradiation in water disinfection was investigated in this study. The use of ultrasound leads to acoustic cavitation. Cavitation is a phenomenon which can be described as the formation, growth and subsequent collapse of microbubbles, occurring in extremely small time interval (milliseconds). In the cavitation process, a large amount of energy is released. The local effects of the cavitation phenomena are locally expressed in the generation of very high temperatures (of the order of 1000–5000 K) and pressures (100–50,000 bar). In addition, cavitation results in the release of free radicals due to pyrolysis of water (Gogate et al., 2003).

The exact mechanism by which cavitation causes inactivation of the microorganisms has not been conclusively established. However, it is believed that it involves a combination of following simultaneously acting mechanisms (Gogate, 2007): (i) Mechanical effects caused by the cavitation phenomena, such as generation of turbulence, liquid circulation currents, and shear stresses; (ii) Chemical effects of cavitation phenomena, including generation of active free radicals; (iii) Heat effects, i.e. generation of local hot spots with very high local temperatures.

It has been recognized that mechanical effects are the most important mechanism for microbial disinfection. Chemical and heat effects play only a supporting role (Mason et al., 2003, Piyasena et al., 2003).

In this study, we investigated the use of ultrasound as an alternative for more traditional techniques like chlorination and UV irradiation for the disinfection of water. A pilot plant was constructed to asses the potential benefits of using ultrasound in comparison with chlorination and UV irradiation for the disinfection of circulating water. The disinfection efficiency of the different techniques was evaluated for a mixed bacterial culture (four bacterial species) in suspension and biofilm. In addition, the operational costs for the various treatments and the pro's and cons of ultrasound were explored.

MATERIALS AND METHODS

Pilot plant setup

A pilot plant was constructed to compare traditional disinfection techniques with ultrasound and was used to asses the potential benefits of using ultrasound in comparison with chlorination and UV irradiation. The installation consists out of four water containers (up to 100 l) with pumps (adjustable water flow of 60 to 1000 l/h), a piping system (100 m), a disinfection unit (chlorination, ultrasound or UV irradiation), and four biofilm monitors with 18 polycarbonate plates on which a biofilm can be formed. On this pilot plant four simultaneous experiments can be conducted. Experiments were conducted in closed loop configuration, with the water circulating through the disinfection unit, the piping system and the water container at 120 l/h.

Bacterial strains, growth conditions, and enumeration

The micro-organisms used in this study are *Escherichia coli, Pseudomonas aeruginosa, Flavobacterium breve* and *Aeromonas hydrophila*. These organisms are selected because of their natural occurrence in a water environment. *P. aeruginosa* is also a known slime former. For preparation of the bacterial suspensions, microorganisms were grown overnight in Brain Heart Infusion broth (BHI) at 30 °C under vigorous aeration and subsequently diluted in tap water to provide a total bacterial concentration of 10^5 CFU/ml.

Bacterial levels were assessed with traditional plate counting on Brain Heart Infusion Agar (BHIA). This is a widely used technique which accurately reflects the number of colony forming units (CFU) remaining after disinfection. Serial dilutions of the treated bacterial suspension and biofilm samples were prepared using 0.9 % sterile saline water

and analysed by a spread plate technique (0.1 ml on BHIA). Following incubation at 30 °C for 24 h the number of colonies is then correlated with the amount of viable colony forming units per ml of suspension or per cm² of biofilm. Bacterial levels were log transformed before statistical analysis (Loper et al., 1984)

Disinfection treatments

In all chlorination experiments the water is chlorinated by adding a stock solution of sodium hypochlorite (31.7 g/l of free chlorine). Dosage of the solution is done continuously, with a membrane pump, in order to obtain a concentration in the treated water of 0.1 mg/l of free chlorine (in a theoretical situation with no consumption of free chlorine). The pH of the treated water is maintained at approximately 7 by adding diluted H_2SO_4 (5 %) when required.

Ultrasound was applied to water using a commercial flow-through system with a reactor volume of 0.98 l (Bandelin). The Sonorex Technik Sonobloc (SB 5.1-1002) consists out of a reactorbloc and an ultrasonic generator (1001 T). The system has a fixed frequency at 25 kHz, a variable power output with a maximum of 1000 W (1020 W/l). The maximum measured value of the electrical consumption is 1040 W.

UV irradiation is applied to water using a flow-through system. The low pressure mercury arc lamp (peak emission at 254 nm) is enclosed in a tubular chamber. A surrounding thin layer of quartz glass shields the lamp from the water that flows parallel to the orientation of the lamps. The UV lamp has a measured electrical consumption of 340 W.

Biofilm experiments

The preventive use of the disinfection techniques against biofilm formation was tested in a closed loop configuration. In a first experiment, nutrient broth was added to the water containers to stimulate bacterial growth. A second experiment was carried out without adding nutrients. Experiments were conducted over a period of 17 days and samples (6 polycarbonate plates per sample) were taken on three different times during this period.

RESULTS AND DISCUSSION

Treatment of suspended bacteria

Water (25 l) inoculated to achieve a final bacterial concentration of 10^5 CFU/ml was circulated and treated in the pilot plant with UV irradiation, chlorination or ultrasound. Bacterial levels were assessed at regular time intervals to investigate bacterial inactivation of the different techniques. It was observed that bacterial levels after three hours of ultrasound treatment are reduced with 2.5 log units (99.7 %), which is comparable to chlorination and UV irradiation (Figure 1). Nevertheless, UV irradiation and chlorination reach their maximal bacterial reduction considerably faster in comparison with ultrasonic treatment.

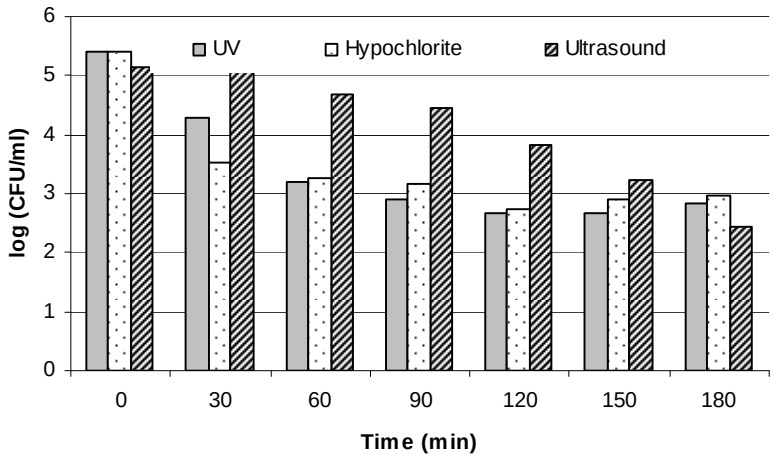

Figure 1: Reduction of the bacterial level of mixed bacterial suspensions by application of UV irradiation, chlorination, or ultrasound. The water volume of 25 l is circulated in a pilot plant and treated for 180 minutes.

When the treated volume is increased to 100 l, the chlorination and UV irradiation are still able to reduce bacterial counts by 99.7% after 3 hours, while ultrasound technology only achieved a bacterial reduction of 81.9 % (data not shown).

Tabel 1: Properties of the disinfection techniques UV irradiation, chlorination and ultrasound applied to disinfect bacteria suspended in circulating water

	UV irradiation	**Chlorination**	**Ultrasound**
Max. reduction (%)	**99.8**	**99.8**	**99.8**
$t_{\text{reduction max}}$ (min)	120	120	180
Energy use (kWh)	1.0	/	2.68
Energy cost (€)	0.15		0.40
Chemicals (mg/l)	/	0.2	/
pH adjustments (ml)	/	0.1	/
Chemicals cost (€)	/	0.00034	/
Operational cost (€)	**0.15**	**0.00034**	**0.40**

The operational costs, calculated as the energy use (0.15 €/kWh) added to the costs of chemicals, of the different techniques are listed in table 1. It is clear that chlorination is economically the most beneficial technique. Ultrasound technology seems to be an energy-demanding technique for water disinfection. When both disinfection efficiency and operational cost are taken into account, chlorination is preferable to UV irradiation and ultrasound. Ultrasound is disfavoured due to the high energy cost and its limitations in the disinfection of larger water volumes.

Prevention of biofilm formation

To analyze the capacity of disinfection techniques to prevent biofilm formation, the pilot plant was used to treat 100 l water circulated through the pilot plant with biofilm reactor. To stimulate biofilm formation, nutrients were added to the water. After 5, 12 and 17 days, samples were taken to monitor the biofilm growth, expressed in CFU/cm^2. It is clear from Figure 2 that without treatment a biofilm is established already after 17 days.

The use of chlorination is able to maintain the bacterial level at log 4 CFU/cm². The application of UV irradiation and ultrasound can reduce the biofilm formation with 1 log unit (90 %) with respect to the control.

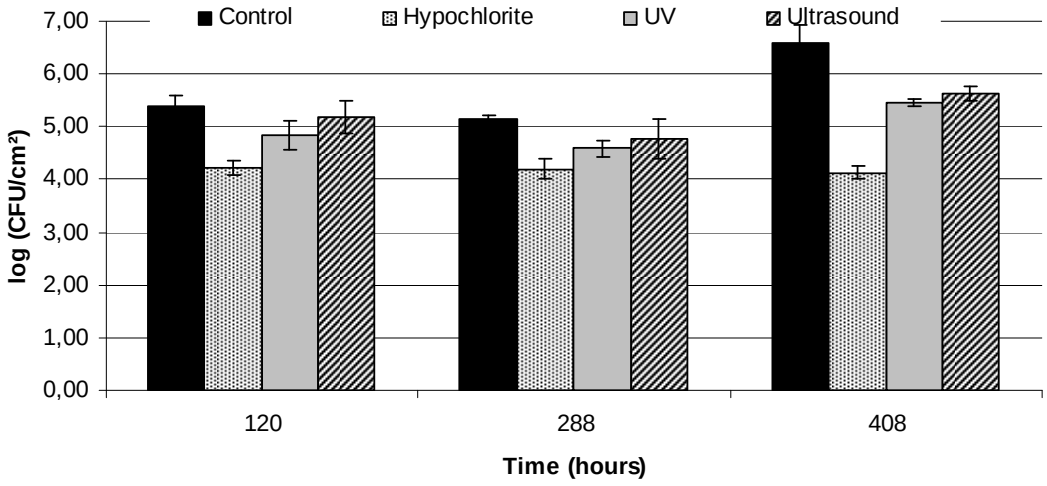

Figure 2: Monitoring of biofilm formation of water with addition of nutrients and the effect of chlorination, UV irradiation and ultrasound on biofilm gowth Biofilm growth is expressed in log (CFU/cm^2). Data are averaged values of 6 samples per sampling moment. The error bars represent standard deviation.

A study by Bott and Tainqing (2004) also investigated the effect of ultrasonic treatment (20 kHz, 600 W) on the formation of biofilm (*Pseudomonas fluorescens*). Over a period of 14 days, the ultrasonic treatment reduced the biofilm accumulation with 70 % in comparison with the control treatment.

To further investigate the prevention of biofilm formation by different disinfection techniques, the experiment was repeated without the addition of nutrients. It was observed that biofilm is readily formed when water is not treated (control) with a disinfection technique (Figure 3). Chlorination on the other hand can entirely prevent biofilm formation as the bacterial counts never exceeded the detection limit. When the water was disinfected using UV irradiation or ultrasound biofilm formation could not be prevented. Nevertheless, a considerable reduction in biofilm is achieved by applying these treatments compared to the control. After 360 hours of treatment with UV irradiation, a reduction of 3.2 log-units (54%) was achieved, compared to the control. With ultrasound treatment a difference of 2.5 log-units (41%) was obtained in comparison with the control.

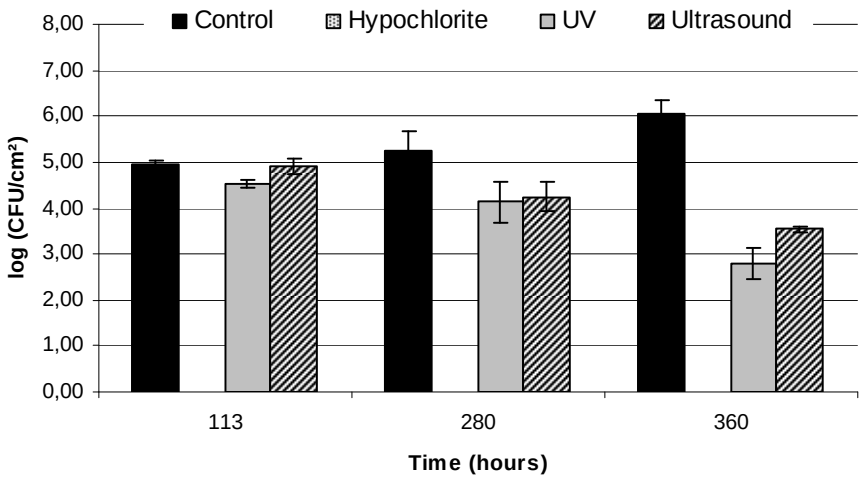

Figure 3: Monitoring of biofilm formation of water without addition of nutrients and the effect of chlorination, UV irradiation and ultrasound on biofilm gowth. Biofilm growth is expressed in log (CFU/cm^2). Data are averaged values of 6 samples per sampling moment. The error bars represent standard deviation.

Based on disinfection efficiency and operational cost (Table 2), the use of chlorination is the most beneficial method to treat bacteria in suspension and in biofilm.

Tabel 2 Properties of the disinfection techniques UV irradiation, chlorination and ultrasound applied to treat water for prevention of biofilm formation

	Control	Hypochlorite	UV irradiation	Ultrasound
Reduction in comparison with control after 360 uur (%)	**0**	**>99.99**	**54**	**41**
Energy use (kWh)	/	/	121	156
Energy cost (€)	/	/	18	23.4
Chemicals (mg/l)	/	2 x 5 ppm / day	/	/
pH adjustments (ml)	/	18	/	/
Chemicals cost (€)	/	0.20	/	/
Operational cost (€)	**/**	**0.20**	**8.7**	**10.8**

Our findings are in agreement with other studies (Jyoti and Pandit, 2001; Blume and Neis, 2004; Jyoti and Pandit, 2004) that compared the operational costs associated with disinfection methods. These studies also showed that the treatment with ultrasonic technology is still an order of magnitude higher as compared to traditional techniques.

CONCLUSION

In this study, a pilot plant was constructed to compare disinfection techniques regarding the operational cost, their efficacy to inactivate suspended bacteria, and the capacity to prevent of biofilm formation. The pilot plant, consisting of water containers containing up to 100 l water and a piping system of 100 m length in total, enabled the study of disinfection in standardized conditions that properly simulate conditions encountered in

industrial processes. Due to the relevance of the scale, our experiments also render information about important upscaling parameters (Hulsmans et al., 2008).

It was shown in this study that, chlorination should be the choice of preference when the efficiency to inactivate suspended bacteria and operational cost are taken into account. The efficacy of UV irradiation and ultrasonic technology to inactivate suspended bacteria are comparable. However, the efficacy of ultrasonic technology decreases when large volumes of water are to be treated. Moreover, compared to UV irradiation and chlorination, ultrasound is a high energy demanding technique.

To prevent biofilm formation, again chlorination appeared to be the most beneficial technique. In the treatment of biofilm the results of UV irradiation and ultrasound are comparable with a slightly higher energy demand for the ultrasonic treatment.

Although chlorination is the preferred technique regarding disinfection efficacy and operational cost, environmental concern has led to the awareness that the use of toxic biocides, like chlorine, should be replaced or reduced. Therefore, it is important that alternative techniques, such as ultrasonic technology should be explored. In this study, it was demonstrated that ultrasonic technology is able to reduce contamination in recirculating water to an acceptable low level. However, due to the high energy demand of the ultrasonic equipment, it may be advantageous to apply ultrasound in combination with another treatment. Considering the fact that ultrasound is able to breakdown bacterial agglomerates, its application may result in an synergetic effect when combined with e.g. chlorination, ozonization, or UV irradiation (Hulsmans et al., in prep.). Process intensification studies or combination of cavitation with other treatment techniques can result in substantial savings as well as quick treatment. Optimization of the operating parameters and further study of combination of cavitation with conventional treatment techniques is required for minimizing the cost of treatment.

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