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ORIGINAL ARTICLE

Determination of the effectiveness of UV radiation as a means of disinfection of metalworking fluids

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Abstract Microbial contamination of metalworking fluids (MWFs) causes biofouling and degradation and is also associated with several health hazards. Development of an effective control method is therefore essential to reduce microbial loading in MWFs. The present study investigated the efficacy and rapidity of UV radiation as a means of disinfection of MWFs under laboratory conditions to determine parameters that could be used to design an in-line UV reactor for enclosed machines. High and low concentrations (10^4–10^7 CFU/mL) of three indicator bacteria, *Pseudomonas fluorescens*, *P. oleovorans* subsp. *lubricantis* and *Mycobacterium chelonae*, were evaluated both as pure cultures and in combinations. The target organisms were irradiated with a high intensity (192 μW/cm^2, 55 W) UV lamp for different exposure time under both static and mixed conditions. For these *Pseudomonas* species with high concentrations of cells under static conditions, only a 56 % reduction was observed within 10 min of exposure, whereas under mixed condition, a 99 % reduction was achieved within 2 min of exposure. In contrast only 74 % reduction was observed for *M. chelonae*. However, with low concentrations of cells under mixed conditions, a 99.99 % and 82 % reduction in viable count was observed for the *Pseudomonas* sp. and *M. chelonae*, respectively. Similar results were observed for mixed culture combinations. Based on these observations high intensity UV in combination with mixing could be successfully used as a means of disinfection of MWFs within a short exposure time and the parameters obtained from the study could be implemented to design a plug flow UV reactor.

Keywords Metalworking fluid · Disinfection · UV · Rapid growing mycobacteria · Pseudomonads

Introduction

Metalworking fluids (MWFs) used in machining systems are complex mixtures of chemicals and are highly susceptible to microbial contaminations (Selvaraju et al. 2011; Saha and Donofrio 2012). The extent of contamination depends on several factors such as age of fluid, type of machining system, and applications of biocides (Virji et al. 2000). Microorganisms in contaminated MWFs not only cause biofouling and degradation of the fluid but are also associated with different diseases such as skin dermatitis and hypersensitivity pneumonitis (Moore et al. 2000; Wallace et al. 2002; Rhodes et al. 2011). In addition, Gram-negative bacteria produce endotoxins that contribute to respiratory illnesses (Selvaraju et al. 2011).

Several methods have been used to reduce microbial contaminations of MWFs. Physical methods such as pasteurization, filtration, centrifugation and dry machining were implemented to control microbial load (Rudnick 2003). Chemical means used for the disinfection process include adding mist suppressants, manufacturing bioresistant MWFs and application of biocides (Klocke and Eisenblatter 1997; Selvaraju et al. 2005). However these control methods have their own disadvantages and limitations. Pasteurization is an energy intensive process as it requires high temperature (42 °C for 30 min) that might cause survival of heat-resistant bacteria, eventually

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giving rise to heat-resistant group of bacteria in the MWF system (Wright 1984). Filtration on the other hand, is successful in removing particulate material but it does not eliminate the cells directly and the microbes have a tendency to grow in filtration systems (Skerlos et al. 2001; Busca et al. 2003; Rudnick 2003). Similarly, chemical methods also have their drawbacks. Biocides cause health hazards such as skin dermatitis when used in high concentrations (Rossmoore 1995; Skerlos et al. 2000). Microorganisms have the capability to hydrolyze biocides and become resistant to them (Virji et al. 2000). Biocides can also breakdown when the metalworking fluid flows through the machining system at high temperature. Due to toxicity involved with biocides they require added handling and disposal cost along with proper applications. Formaldehyde-based biocides also cause occupational asthma in workers (NIOSH 2001). Bio-resistant metalworking fluids lose their properties with time and eventually fails to prevent microbial contamination. Mist suppressants, being sensitive to shear effects, lose their chemical properties with time (Rudnick 2003).

In order to overcome the disadvantages associated with the physical and chemical disinfection methods for MWFs, researchers have implemented non-ionizing radiation such as ultraviolet (UV) (Peppiatt and Shama 2000; Johnson and Phillips 2002; Coogan 2005). There are several advantages of UV over other disinfection methods. It does not produce any undesirable by-product, is independent of pH and temperature, and requires less contact-time compared to other methods such as chlorination (Chang et al. 1985; Katara et al. 2008). It is also cost-effective because it does not require additional storage and disposal after use. UV has been used successfully in the disinfection of water (Severin 1980) and inactivation of endotoxin (Anderson et al. 2003).

Previous studies on UV disinfection of MWFs have proved to be an effective means of reducing bacterial contamination (Peppiatt and Shama 2000; Johnson and Phillips 2002; Coogan 2005). However, in earlier studies, the targeted decline in cell numbers required longer contact times because of the turbidity and opacity of the MWF that block UV penetration and cause shielding effects. Coogan (2005) reported only 10 % or less inactivation of microorganisms per pass of the contaminated fluid; higher level inactivation was achieved with more passes per unit time. Sterilization of MWF is not feasible as machining processes are continuously exposed to microbial contamination from different sources such as water and biofilms present in the sumps (Moore et al. 2000; Lucchesi et al. 2012). Therefore, control methods should be designed to reduce microbial load instead of sterilization of MWFs. Based on these facts a 2-log reduction was targeted in this study. In general, the levels of microbial contamination in MWFs range from 10^4 to 10^{10} colony forming units (CFU)/mL (van der Gast et al. 2001; Saha and Donofrio 2012). Therefore, if applied at the earlier stages of contamination (below or around 10^4 CFU/mL), UV might be effective in achieving the targeted reduction in microbial counts within a shorter exposure time.

In contrast to previous studies (Peppiatt and Shama 2000; Johnson and Phillips 2002; Coogan 2005), the proposed plug flow UV reactor could be implemented as an in-line UV system in small enclosed machining operations. Enclosed machining systems not only reduce exposure of MWFs to workers but also prevent contaminants from entering MWFs (Ross et al. 2004), thus increasing the effectiveness of UV irradiation as a control method. The UV bioreactor will be non-invasive and will be placed at several places in the MWF system such as the point of entry and exit of the sump. Under laboratory conditions the design of the UV experiment was based on static conditions where the height of the solution in the Petri dish was equal to the radius of the MWF tubing system used commonly in enclosed machining systems. In a static condition the point of radius is the position that will be least hit by the UV irradiation thus offering shielding effect to the bacterial cell lying in that position. However, under mixed flow individual bacterial cells will not remain in a fixed position and will not be affected by the shielding effect as mixing will maximize the exposure of the cell to the UV irradiation. By implementing the UV reactor as an in-line UV system at several points in a MWF system, the disinfection process can be maximized and the exposure time reduced.

The overall objective of the present study was to investigate the effectiveness of UV irradiation as a means of disinfection of MWFs that will enable the determination of design parameters such as intensity of UV lamp, contact time, distance of the UV lamp and mixing for the development of an in-line UV reactor to control microbial load in enclosed machining systems. Since species of *Pseudomonas* are commonly found in contaminated MWF, *P. fluorescens* and *P. oleovorans* subsp. *lubricantis* (Saha et al. 2010) recovered from the samples used in the previous study were used as the indicator organisms. In addition, *Mycobacterium chelonae* was also selected because it represents rapidly growing mycobacteria (RGM), which are potential pathogens associated with several health hazards (Selvaraju et al. 2005; Rhodes et al. 2011).

Materials and methods

MWF samples

Two different unused semi-synthetic MWF samples (MWF-A, MWF-B) from two different manufacturers were used for the UV exposure study. The MWF-A had a blue dye and MWF-B was without a blue dye. The manufacturers use different colored dyes to separate one product from another.

Apart from adding coloration the dye did not add any other properties to the MWFs.

Bacterial strain

Three indicator bacteria, *P. fluorescens*, *P. oleovorans* subsp. *lubricantis* and *M. chelonae* were used in the study. The *Pseudomonas* species were recovered as part of a previous study from used MWFs (Saha et al. 2010) and the *M. chelonae* culture was obtained from the Michigan State Laboratory. *P. fluorescens*, *P. oleovorans* subsp. *lubricantis* and *M. chelonae* were maintained on *Pseudomonas* isolation agar (PIA) (BD, Franklin Lakes, NJ) , tryptic soy agar (TSA) and Middlebrook 7H11 agar (M7H11) (Remel, Lenexa, KS), respectively. The incubation temperature of *P. fluorescens* was 30 °C and *P. oleovorans* subsp. *lubricantis* and *M. chelonae* was 35 °C.

Survivability and culturability of bacteria in freshly diluted MWF sample

Diluted (5 %) MWFs have a high pH range of 9.2–10.0 due to their chemical composition (Rabenstein et al. 2009). The two unused MWFs used in this study had a pH of 9.0–9.2. To determine the effect of pH on *P. fluorescens*, the MWF was diluted (5 %) with sterile deionized water or with sterile phosphate buffer (pH 7.0). The phosphate buffer was used to neutralize the pH of the MWF solution as contaminated MWFs have pH in the range of 6.8–7.3 (Rabenstein et al. 2009). Comparative death-rates were calculated with the bacterium spiked in MWF, separately diluted with water and phosphate buffer (pH 7.0) using the formula: $kt=\ln(N_0/N_0-N_d)$ where N_d = final bacterial concentration, N_0 = initial bacterial concentration; k = death rate and t = exposure time.

UV exposure study

UV germicidal lamps (254 nm) with intensities of 38 μw/cm^2 (15 W) and 192 μw/cm^2 (55 W) (Sylvania, Danvers, MA) were used to study the effect of UV irradiation on different bacteria spiked in freshly prepared (5 % diluted) MWF. The 5 % dilution was used in the study to simulate the dilution used in different machining operations in industries (Barr 1998; Johnson and Phillips 2002). The dilution was prepared by using sterile phosphate buffer (pH 7.0). The experimental parameters such as the amount of solution (6.75 mL) used in the study, were determined from the diameter of the pipe (0.318 cm) through which MWF typically flows in an enclosed machining system such as in grinding and milling operations. For these systems, the UV reactor would be implemented as an in-line system by using the formula $V=\Pi r^2h$, where V = volume of MWF solution, r = radius of petri dish used in the exposure study and h = height of the fluid in the Petri dish which is equal to the radius of the tubing system. Under laboratory testing conditions the MWF samples were exposed to UV from one side of the MWF samples the height of the fluid in the petri dishes was equal to the radius of the tubing system. The distance of the UV lamp from the fluid was 4 mm. The UV exposure study was conducted using both stagnant and turbulent conditions by taking the calculated volume of solution spiked with the bacteria of interest in a petri dish of diameter 5.2 cm. The static condition represented laminar flow (without mixing) where the spiked bacterial cells would always be at the same depth with respect to the UV irradiation. Mixing in the solution was created using a magnetic spin bar rotating at 150 rpm. The reduction of the bacterial count was determined and recorded for each type after UV exposure by performing serial dilution in 0.85 % physiological saline and spread plating on different bacteriological media, i.e., PIA for *P. fluorescens* MWF-1 (30 °C), TSA for *P. oleovorans* subsp. *lubricantis* (35 °C) and M7H11 for *M. chelonae* (30 °C). After incubation for 24–48 h the CFU/mL was determined for each exposure time and the reduction in the bacterial load was determined. The dosage was calculated using the formula $D=I\times T$, where D = dosage; I = intensity of the lamp; T = exposure time (US EPA 2006). The experiments were conducted three times and were performed in random order.

Standardized collimated beam experiment

A bench-scale collimated beam experiment was performed at NSF International, Ann Arbor, MI, according to the NSF International and American National Standard (ANSI) protocol (NSF/ANSI 55, 2007) to determine the dose response of *P. fluorescens* MWF-1 in MWF using an UV lamp of 129 μW/cm^2 intensity. The summary of the experiment is presented in Table 1. The standardized collimated beam experiment is routinely carried out to develop dose response curve for different microorganisms to determine the disinfection efficacy of drinking water treatment systems.

Results and discussion

Survivability of *P. fluorescens* in metalworking fluid

The survivability of the indicator organism in absence of UV radiation in MWF was studied. The purpose of this experiment was to determine the effect of the MWF matrix on the tested bacteria over the experimental exposure time, so that the results reflect solely the effects induced by UV. When inoculated separately in physiological saline and MWF diluted with sterile distilled water, *P. fluorescens* exhibited a difference in viable count (CFU/mL). As *P. fluorescens* cannot grow well at a higher pH, it was suspected that the decline could be due to

Table 1 Summary of standardized collimated beam experiment using 5 % diluted metalworking fluid (MWF)

Incident irradiance (I_0)	129 μw/cm^2				
Average irradiance (I_a)	36.7 μw/cm^2				
Absorbance (abs)	4.55 at 254 nm				
Transmittance	0.0000316 (10^{-abs})				
% Minimum dose	Dose[a] (mJ/cm^2)	Exposure time (s)	*P. fluorescens* concentration	Average concentration	Log reduction
0	0.0	0	1.20E+07	1.23E+07	0
0	0.0	0	1.25E+07		
0	0.0	0	1.25E+07		
30	9.0	245	8.00E+06	8.10E+06	0.2
30	9.0	245	8.20E+06		
30	9.0	245	8.10E+06		
45	13.5	368	4.50E+06	4.47E+06	0.4
45	13.5	368	4.40E+06		
45	13.5	368	4.50E+06		
60	18.0	490	2.00E+06	2.03E+06	0.8
60	18.0	490	2.10E+06		
60	18.0	490	2.00E+06		
75	22.5	613	9.20E+05	9.07E+05	1.1
75	22.5	613	9.00E+05		
75	22.5	613	9.00E+05		

[a] Dose=Exposure time×I_a

high pH (9.2) of the unused MWF. The optimum pH for growth of *P. fluorescens* is reported to be 6.0–7.0 (Buchanan and Bagi, 1999). To confirm this, survivability of *P. fluorescens* was studied by inoculating the bacterium in MWF diluted with sterile water or phosphate buffer (pH 7.0). The death rate was more in case of MWF diluted with sterile water (k=−6.30 h^{-1}) than phosphate buffer (k=−2.50 h^{-1}). A similar study conducted by Havel (2002) to determine any inhibiting effect of MWF matrix on the bacteria in absence of UV radiation observed 70 % to 90 % decrease in viable counts within 60 min (duration of trials). Based on these observations the MWF was diluted with phosphate buffer for further UV exposure studies.

Standardized collimated beam experiment

In the collimated beam experiment the average UV irradiance due to absorbance (4.55 units at 254 nm) by 5 % diluted MWF was determined to be 36.7 μW/cm^2. Thus it was evident that there was a high absorbance of UV by the MWF due to its chemical composition and viscosity; only a 1-log reduction in viable count was achieved for a dosage of 22.5 mJ/cm^2 with an exposure time of 10.2 min. The regression equation obtained by plotting dosage against log reduction (Fig. 1) indicated that to obtain a 2-log reduction of viable cell count a dosage of 42.9 mJ/cm^2 was required. Therefore, in order to achieve the 42.9 mJ/cm^2 dosage in shorter exposure time (2–3 min), a high intensity UV lamp had to be employed.

UV exposure study

UV irradiation of P. fluorescens under static condition

An initial study was performed with *P. fluorescens* using a low intensity UV lamp (38 μW/cm^2 or 15 W), which was not sufficiently effective for the desired 2-log reduction within a short exposure time. After 30 min of exposure only 79.2 % decline in the viable count was achieved. Thus, for future UV experiments a germicidal lamp of 55 W with 192 μW/cm^2

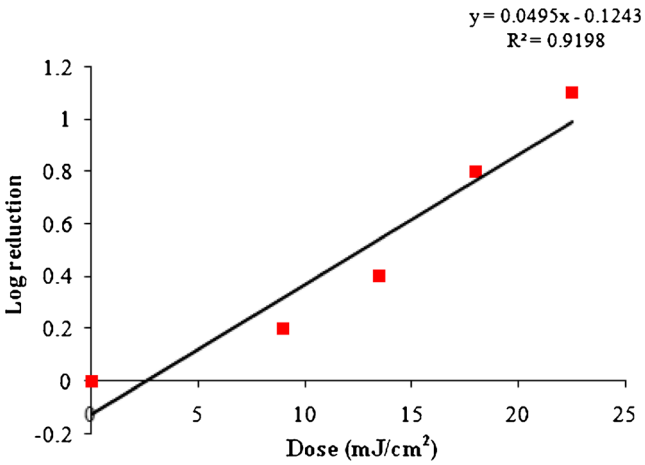

Fig. 1 Collimated beam experiment with *Pseudomonas fluorescens* in metalworking fluid (MWF) to determine the relationship between dosage and Log reduction of viable cell count

intensity was used to obtain a 99.0 % reduction in the microbial count. A positive control experiment was conducted with physiological saline (0.85 % NaCl) to determine the effectiveness of the UV lamp (55 W). A 2.8-log (99.8 %) reduction in bacterial count was observed within 1 s of UV exposure, indicating the effectiveness of the lamp.

UV irradiation of P. fluorescens in MWF (MWF-A and MWF-B) under static condition using the 55 W lamp

P. fluorescens in MWF-A was irradiated with 55 W UV lamp under approximate laminar flow conditions. After 10 min of exposure a 56.0 % reduction in viable cell count with a death rate of $-8.32\ h^{-1}$ was observed (Fig. 2). It was assumed that the viscosity of MWF along with the blue dye present in MWF-A was providing a shielding effect to the cells suspended in the fluid, as it is well known that UV has poor penetration capability in opaque and colored solutions compared to clear samples (Vieira et al. 2007). Therefore, further experiments were conducted with MWF-B (without the blue dye) to investigate the effectiveness of UV irradiation. After 10 min of UV exposure the reduction in bacterial cell count was only 74.0 % in MWF-B (Fig. 3), which clearly indicated that even in absence of the blue dye, a strong shielding effect from the MWF is persistent, which could be attributed to the chemical composition of the fluid (Rabenstein et al. 2009). However, the death rate ($k = -8.32\ h^{-1}$) of the bacteria in MWF without the blue dye was more than the death rate ($k = -5.10\ h^{-1}$) of the microbe in MWF with the blue dye.

UV irradiation of P. fluorescens and P. oleovorans subsp. lubricantis in MWF-B with mixing

In an attempt to obtain a 2-log reduction within a short exposure time, mixing was introduced using a magnetic stir bar, in the

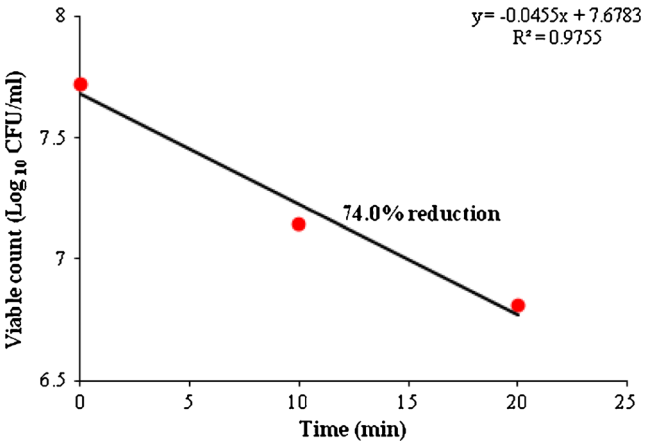

Fig. 3 Reduction of *P. fluorescens* spiked in 5 % diluted MWF with UV irradiation (55 W) without blue dye under approximate laminar flow conditions. Data presented as mean of three experimental trials. All standard deviation (±SD) calculated were <0.02

experimental design where *P. fluorescens* in MWF-B solution was exposed to UV at 1-, 2- and 3-min time intervals. In contrast to static conditions, after 2 min of UV exposure a 98.8 % reduction in the bacterial viable cell count was observed (Fig. 4). The corresponding death rates are presented in Table 2. Thus, a 2-log reduction with much shorter exposure time can be achieved with the optimization of the mixing condition in the experiment. The calculated dosage (D) for *P. fluorescens* in MWF-B under mixing was found to be 23.0 mJ/cm^2, which was lower than the estimated (42.9 mJ/cm^2) dosage determined from the collimated beam experiment, to obtain a 2-log reduction.

A similar experimental design was applied to *P. oleovorans* subsp. *lubricantis* in order to investigate the feasibility of the design with different bacteria. Exposure of *P. oleovorans* subsp. *lubricantis* to UV irradiation under mixed condition in MWF-B resulted in 98.9 % reduction in 2 min (Fig. 4). The

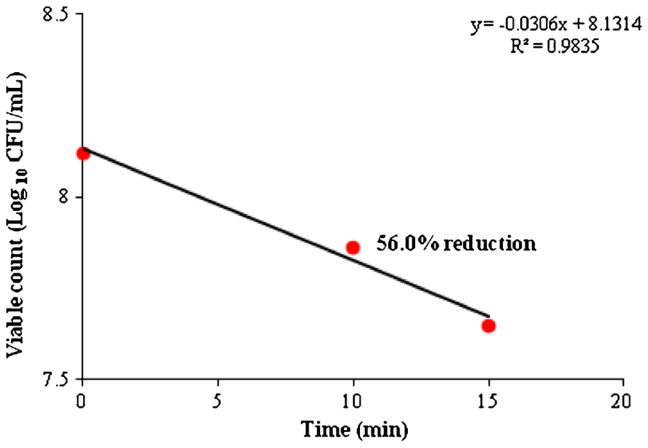

Fig. 2 Reduction of *P. fluorescens* spiked in 5 % diluted MWF with UV irradiation (55 W) with blue dye under approximate laminar flow conditions. Data presented as mean of three experimental trials. All standard deviation (±SD) calculated were <0.04

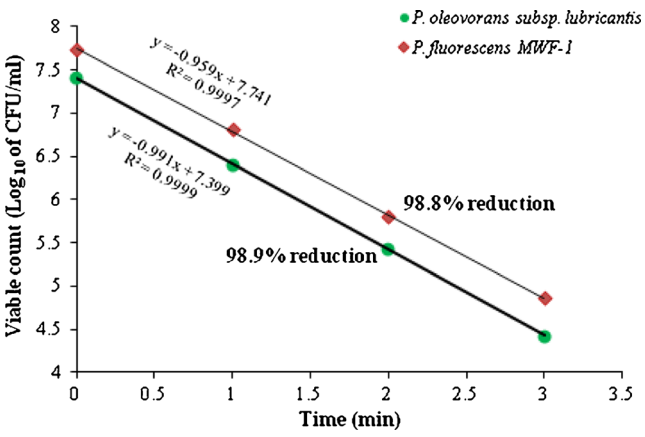

Fig. 4 Reduction of *P. fluorescens* and *P. oleovorans* subsp. *lubricantis* spiked in 5 % diluted MWF with UV irradiation (55 W) using mixing. Data presented as mean of three experimental trials. All standard deviation (±SD) calculated were <0.02

Table 2 Effects of UV radiation on three indicator bacteria in two different semi-synthetic metal working fluids (MWFs). Values of initial and final viable counts represent mean of six replicates ($n=6$)

Bacteria	UV lamp intensity ($\mu W/cm^2$)	MWF sample[a]	Condition	Initial viable count (CFU/mL)	Final viable count (CFU/mL)	Exposure time (in h)	Death rate (k, h^{-1})
P. fluorescens	38	MWF-A	Static[b]	$3.9(\pm0.05)\times10^7$	$1.92\ (\pm0.04)\times10^7$	0.25	−4.43
P. fluorescens	192	MWF-A	Static	$5.3(\pm0.05)\times10^7$	$1.4\ (\pm0.02)\times10^7$	0.16	−8.32
P. fluorescens	192	MWF-B	Mixing	$5.35(\pm0.2)\times10^7$	$6.4\ (\pm0.01)\times10^5$	0.03	−27.70
P. oleovorans subsp. *lubricantis*	192	MWF-B	Mixing	$2.5(\pm0.005)\times10^7$	$2.73\ (\pm0.01)\times10^5$	0.03	−28.24
M. chelonae	192	MWF-B	Mixing	$3.1(\pm0.1)\times10^4$	$5.50\ (\pm0.2)\times10^3$	0.03	−10.81

[a] Unused 5 % diluted MWF from two different industries were used

[b] Approximate laminar flow condition

corresponding death rates (k) values are presented in Table 2. This clearly indicated that in the process of UV disinfection of MWF, solely the use of simple UV irradiation is not sufficient to achieve a 2- log reduction within shorter exposure time. In order to achieve a 99.0 % reduction in cell count, a combination of UV light with mixing is necessary to implement in the design of the in-line UV bioreactor system because MWFs offers a strong shielding effect owing to its complex chemical composition and viscosity.

UV irradiation of P. fluorescens and P. oleovorans subsp. lubricantis with different cell concentrations

It was important to study the effectiveness and feasibility of UV irradiation on different cell concentrations because varying levels of bacteria are found in contaminated MWFs (Virji et al. 2000). Different cell concentrations (10^4, 10^6, and 10^7 CFU/mL) of *P. fluorescens* and *P. oleovorans* subsp. *lubricantis* were exposed to UV irradiation for 15, 30, 60, 120 s. The targeted 2-log reduction was obtained at 60 s for 10^4 CFU/mL and 120 s for 10^6 and 10^7 CFU/mL. The percentage reduction in viable cell counts at 10^4, 10^6 and 10^7 CFU/mL and the equivalent values of the death rates are presented in Table 3.

UV disinfection of Mycobacterium chelonae in MWF-B with mixing

The levels of contamination of RGMs such as *M. immunogenum* and *M. chelonae* found in used MWFs are reported to be lower than the pseudomonads (Shelton et al. 1999). Thus, in this study, 10^4 and 10^6 CFU/mL of *M. chelonae* were used to investigate the efficacy of UV irradiation (Table 3). Compared to the *Pseudomonas* species, a lower level of cell reduction was observed in case of *M. chelonae* under similar conditions (Fig. 5), which could be attributed to multiple factors such as cell wall composition and dark repair of DNA damage. *Mycobacteria* have high concentrations of peptidoglycan and mycolic acids that could provide resistance to UV irradiation (Jarlier and Nikaidi 1990; Nikaido et al. 1993). Also, mycobacteria have longer generation times that give them a significant opportunity for dark repair of UV damages caused to the nucleic acid (LeChevallier 2004). The results of the exposure studies indicated that, even with low density of cells, a higher dosage of UV is required for a 2-log reduction of RGMs.

UV irradiation of mixed cultures in MWF-B with mixing

In machining systems, used MWFs contain different species of bacteria. In an attempt to simulate the condition of used MWF, a mixed culture of *P. fluorescens* and *P. oleovorans* subsp. *lubricantis* were exposed to UV for 1 and 2 min. Both of these bacteria responded similarly in that a 2-log reduction was obtained within 2 min of exposure. When a mixed culture of *P. oleovorans* subsp. *lubricantis*, *P. fluorescens* and *M. chelonae* was exposed to UV irradiation for 0, 2 and 3 min, the pseudomonads again exhibited a 2-log reduction (*P.*

Table 3 UV exposure study of three indicator bacteria with different levels of initial viable counts

Bacteria	Initial viable counts (CFU/mL)[a]	Death rate ($k\ h^{-1}$)	% Reduction (within 2 min)
P. fluorescens	10^4	−62.61	99.99
	10^6	−32.53	99.5
	10^7	−27.70	99.0
P. oleovorans subsp. *lubricantis*	10^4	−64.05	99.99
	10^6	−33.97	99.6
	10^7	−28.24	99.0
M. chelonae	10^4	−10.81	82.3
	10^6	−8.50	74.2

[a] Initial viable counts of each bacterium in colony forming units (CFU/mL)

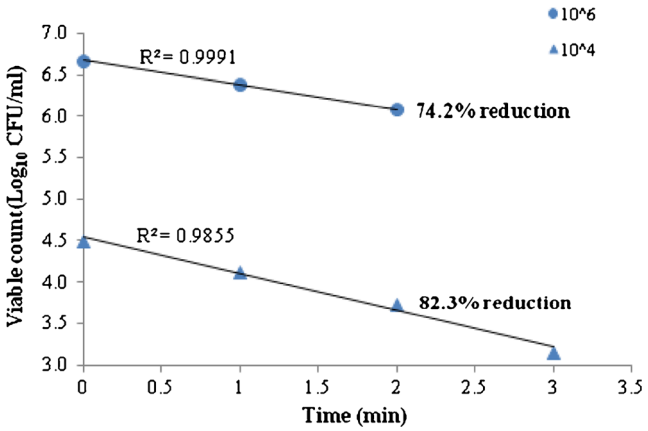

Fig. 5 Reduction in the number of viable cells of *M. chelonae* in MWF with two different cell concentrations (10^4 and 10^6 CFU/mL) exposed to UV irradiation (55 W). Data presented as mean of three experimental trials. All standard deviation (±SD) calculated were <0.01

fluorescens 99.2 %, *P. lubricans* 99.0 %) after 2 min of exposure, but only 74.1 % reduction in viable cell count was observed in the case of *M. chelonae*. However, after 3 min of irradiation, the cell count of *M. chelonae* was reduced by 97.4 % (close to 2-log reduction) (Fig. 6).

Previous studies using UV irradiation as a control method required a longer exposure time to control microbial contamination such as 2-log reduction in 60 min for *P. fluorescens* (Johnson and Phillips 2002) and 4-log reduction in 6–8 h for industrial contaminated MWF samples (Peppiatt and Shama 2000). Coogan (2005) reported that a combination of high flow rate and turbulent flow was required to maximize transport into and out of the irradiated kill zone. Similarly, a study on biocides conducted by Selvaraju et al. (2005) reported less than 2- log reduction in bacterial load within 15 and 30 min of contact time for *P. fluorescens* and *Mycobacterium*,

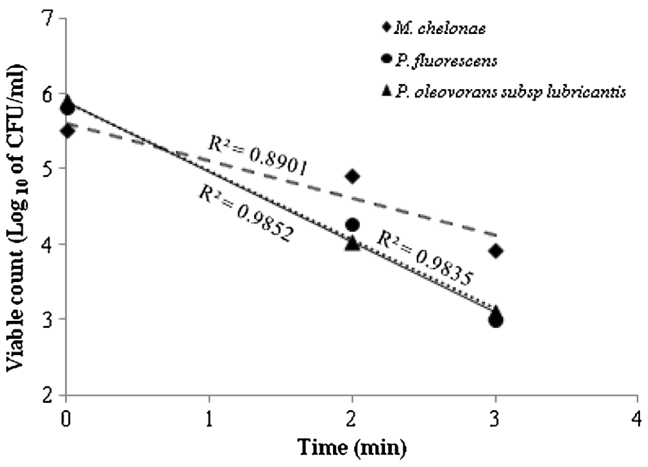

Fig. 6 Reduction in the number of viable cells of mixed culture of *P. fluorescens*, *P. oleovorans* subsp. *lubricantis* and *M. chelonae* exposed to UV irradiation (55 W). Data presented as mean of three experimental trials. All standard deviation (±SD) calculated were <0.23

respectively, using formaldehyde and non-formaldehyde based biocides at different concentrations. It was also observed that the mixed culture of *P. fluorescens* and *Mycobacterium* was less susceptible to the biocides, compared to pure cultures. However, in the present study 2-log reduction was achieved within 2 min of UV exposure for MWF inoculated with both pure and mixed cultures. Based upon these findings, the experimental design with mixing and high intensity UV lamp indicates the feasibility of using these parameters to design an in-line plug flow UV reactor in which, baffles will be incorporated to create the mixing of the MWF to achieve the targeted 2-log reduction.

Conclusion

The results of the UV exposure studies demonstrated that UV irradiation could be efficiently utilized as a means of disinfection of contaminated MWFs. In industry, the effectiveness of such experimental designs depend upon achieving a greater reduction in microbial load within a short time of exposure, based upon which the present target was to obtain a 2-log reduction within the shortest possible exposure time. The results demonstrated that a combination of high intensity UV lamp and mixing could successfully diminish the exposure time to achieve 2-log reduction. Based on the UV exposure study with mixed cultures of *P. fluorescens*, *P. oleovorans* subspecies *lubricantis* and *M. chelonae*, a minimum dosage of 35 mJ/cm^2 is recommended to obtain an overall 2-log reduction. Also, the use of UV could successfully replace biocides applied to control microbial contamination, considering the health hazards associated with such chemical disinfection methods. The next stage would be to conduct a pilot study utilizing the obtained results and parameters. It is also recommended to design a plug flow UV reactor based on the design parameters such as high intensity UV lamp, quartz tube with diameter of the MWF tubing system, position of the UV lamp and mixing of MWF using baffles.

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