Response of Different Strains of *Enterococcus faecalis* to UV Inactivation after Freezing

W. Gao and A. Williams

Abstract—Response of two strains of *Enterococcus faecalis* bacteria, ATCC strain 29212 and ATCC strain 51299 (vancomycin resistant) to UV inactivation after survived freezing treatment was examined. The test microorganisms were frozen at -7 °C, -15 °C or -30 °C with one, three or five freeze and thaw cycles prior to UV irradiation to investigate the effect of freezing temperature and freeze thaw cycles on the efficacy of UV inactivation. Experimental results suggest that freezing influenced the response of *Enterococcus faecalis* cells to UV irradiation. Freezing treated cells behaved differently compared to those had not frozen.

Index Terms—*Enterococcus faecalis*, freezing treatment, UV disinfection, waterborne pathogens, water and wastewater treatment.

I. INTRODUCTION

Conventional municipal wastewater treatment systems can significantly reduce the population of microorganisms; however, after treatment wastewater treatment plant (WWTP) effluents may still contain high numbers of fecal bacteria. Published research data indicated that the portion of pathogenic and antibiotic resistant bacteria in WWTP effluent remained at significant concentrations or even increase [1]-[5]. Release of pathogenic and antibiotic resistant microorganisms with WWTP effluents is a growing public health concern [6], [7]. Multiple antibiotic resistant fecal coliforms and enterococci including vancomycin resistant *Enterococcus* (VRE) have been detected in influent, effluent, and sludge from municipal wastewater treatment plants [8-9]. WWTPs provide a favorable environment for gene transfer due to high bacterial densities and nutritional richness of the influents [4], [8], [9]. Applications of UV disinfection in water and wastewater treatment systems have increased in recent years in Canada. Numerous research has been carried out to find the correlations between water quality, bacterial sensitivity and UV disinfection efficiency [10]-[12], the impact of cold temperatures on UV disinfection processes, especially, the responses of the pathogenic microorganisms after experiencing freezing to UV is however seldom examined [13], [14]. As a treatment alternative, natural or mechanical freezing has been used for various water or wastewater treatment, sludge pre-treatment or conditioning, and desalination [15]-[19]. Depending on the requirements of the finished water quality, UV disinfection may be needed after freezing treatment. Waterborne microorganisms might behave differently to UV

irradiation after survived freezing compared to those without experience freezing [13], [14], [20]. A recent study by Williams et al. [14] revealed that after freezing waterborne microorganisms such as *Escherichia coli*, *Enterococcus* and *Bacillus* behaved differently to UV irradiation compared to those had not frozen. Freezing affected the response of these microbes, a lower UV inactivation level was observed in the freezing treated bacteria in general. Furthermore, freezing also had an impact on the level of photoreactivation of *Escherichia coli*, *Enterococcus* following UV irradiation [21]. Obviously lower levels of photoreactivation were observed in the freezing treated *E. coli* and *Enterococcus* cells. The experimental results suggested that less photoreactivation could be expected if freezing is used as a treatment method prior to UV disinfection. The effect of freezing on the ability of the test microbes to photoreactivate seems to be strain and species dependent. This study was carried out to examine the response of two different strains of *Enterococcus faecalis* bacteria: ATCC strain 29212 and ATCC strain 51299 (vancomycin resistant) to UV inactivation after freezing under different temperatures with one, three or five freeze thaw cycles. *Enterococcus faecalis* was selected because they are one of the predominant species in water environment [22].

II. MATERIALS AND METHODS

A. Test Microbes and Cell Solution Preparation

The strains of *E. faecalis* bacteria used in this study were ATCC strain 29212 and ATCC strain 51299. Both of the *E. faecalis* strains are considered to be opportunistic pathogenic. The procedures used by [23] were followed for cell solution preparation. The ATCC strain 51299 was vancomycin resistant. Vancomycin (Sigma-Aldrich Inc., St. Louis, MD, USA) was added to the growth medium (TSB) of *Enterococcus faecalis* ATCC 51299 to a final concentration of 4 µg/mL to ensure that the resistance did not diminish. The cell solution concentration was approximately 1×10^8 CFU/mL.

B. Freezing Treatment and UV Irradiation

The same freezing treatments and UV inactivation procedures reported by [13] were used in this study. Two hundred mL of stock solution at room temperature (20 °C) were frozen at -7 °C, -15 °C or -30 °C in a walk-in freezer (Climate Testing Systems Incorporated, Warminster, PA, USA). The temperature fluctuation for the freezer was $\pm 0.5^\circ\text{C}$. After freezing for 24 hours, samples were removed and thawed in a digital Isotemp 228 water bath (Fisher Scientific Ltd., Whitby, ON, Canada) set at 20°C. It took

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about 1 hour for the samples to melt.

Once the samples completely melted, UV treatments started. Freeze thaw cycles of 1, 3 and 5 were used to examine how repeated freezing and thawing could affect the response of the test microbes to UV irradiation. A UV collimated beam device with a low pressure mercury vapour lamp (monochromatic at 253.7 nm) (Trojan Technologies, London, ON, Canada) was used as a UV source. UV fluences of 6, 8, 20 and 25 mJ/cm² were used to get 1 to 4 log reduction of cell concentration. More detailed information about the experimental setup could be found in [13]. Cells were frozen prior to UV inactivation and their response to UV inactivation was compared with those without freezing treatment. All experiments were replicated at least three times. The Log inactivation of the test microorganisms was calculated as:

$$\text{Log inactivation} = \log_{10} \left(\frac{N}{N_0} \right)$$

where N_0 = the concentration of microorganisms (in CFU/mL) of the non-irradiated sample, and N = the concentration of microorganisms able to reproduce after irradiation.

III. RESULTS AND DISCUSSION

Fig. 1 compares the average UV inactivation levels of the two strains of *E. faecalis* bacteria that with and without freezing treatment (the controls). For the freezing treated cells, the data presented in Fig. 1 are the average values obtained by combining the data under all freezing temperatures and freeze thaw cycles. Freezing influenced the behavior of *E. faecalis* to UV inactivation. In general, lower log inactivation levels were observed in the freezing treated cells for both strains of *E. faecalis* compared to those had not frozen. After freezing *E. faecalis* cells became less sensitive to UV irradiation. The control samples had about 0.3 to 0.4 more log inactivation than those freezing treated ones.

Fig. 1. Response of difference strains of *E. faecalis* to UV inactivation: comparison of the freezing treated samples with those without freezing (the controls).

Fig. 2 (a, b, c) illustrates the log inactivation levels of the freezing treated *E. faecalis* obtained under a given temperature and UV fluence in comparison to those of the controls. It was observed under all three freezing temperature conditions that higher UV inactivation levels were obtained

for the cells that had not frozen as compared to the freezing treated ones, especially at lower UV fluences (6 and 8 mJ/cm²). Between the two strains of *E. faecalis*, ATCC strain 51299 (vancomycin resistant) had smaller differences in log inactivation levels between the control and the freezing treated ones in most cases except at -30 °C while the reduction in UV inactivation level was slightly more noticeable in the freezing treated cells of ATCC strain 29212. Overall, the extent of reduction in UV inactivation efficiency seems varied with strain type, freezing temperature and UV fluences. As UV fluence increased, the difference between the control and the freezing treated cells or between the two strains became less noticeable. It is expected because cells would be killed after exposure to high strength of UV light whether or not they were pre-exposed to freezing.

Fig. 2. Log inactivation levels of the freezing treated and nonfrozen *E. faecalis* cells under a given UV fluence and temperature: (a) freezing treated cells were frozen at -7 °C, (b) freezing treated cells were frozen at -15 °C, (c) freezing treated cells were frozen at -30 °C (co-29212 = control of ATCC strain 29212; ft-29212 = freezing treated ATCC strain 29212; co-51299 = control of ATCC strain 51299; ft-51299 = freezing treated ATCC strain 51299).

Table I summarizes the percentage decrease in the level of log inactivation of freezing treated samples compared to their corresponding control samples under a given freezing temperature and UV fluence. For the ATCC strain 51299 (vancomycin resistant) of *E. faecalis*, the most obvious reduction in log inactivation level was observed at UV fluence of 6 mJ/cm² after frozen at -30 °C, there were 60%

fewer freezing treated ATCC 51299 cells were inactivated compared to the controls. It was not clear why ATCC strain 51299 cells frozen at -30 °C were so resistant to UV inactivation. The most reduction in the UV inactivation level for the ATCC 29212 strain occurred after freezing at -7 °C followed with UV irradiation at fluence of 6mJ/cm²; the reduction in log inactivation level reached 32.3%. If considering all UV fluences and freezing temperatures examined in this study, the average reduction in UV inactivation level after freezing was 8% to 16% for ATCC strain 29212, and 8% to 36% for ATCC strain 51299. Freezing temperature alone had no obvious influence on the level of UV inactivation of the freezing treated cells. Cell frozen at -7 °C, -15 °C or -30 °C all responded similarly to UV irradiation. Freeze thaw cycles as a factor also had no obvious impact on the UV inactivation levels of freezing treated cells.

TABLE I: % REDUCTION IN LOG INACTIVATION OBSERVED IN THE FREEZING TREATED CELLS COMPARED TO THE CONTROL SAMPLES

UV Fluence (mJ/cm ²)	Freezing Temperature (°C)					
	-7		-15		-30	
	ATCC 29212	ATCC 51299	ATCC 29212	ATCC 51299	ATCC 29212	ATCC 51299
6	32.3	18.9	25.9	28.2	20.3	60.0
8	19.3	15.6	15.7	18.6	7.7	55.6
20	6.1	-1.2	2.4	-5.5	-2.9	13.7
25	6.5	0.3	2.2	-2.9	8.0	16.5

IV. CONCLUSION

Freezing influenced the response of both ATCC strain 29212 and ATCC strain 51299 (vancomycin resistant) of *E. faecalis* cells to UV irradiation. Overall *E. faecalis* bacteria became less sensitive to UV inactivation after freezing. The effect of freezing on the response of *E. faecalis* to UV seems to be strain dependent. UV inactivation effect was reduced significantly under certain conditions after *E. faecalis* cells survived freezing. No significant difference in UV inactivation was observed for the cells frozen at -7 °C, -15 °C or -30 °C. Freeze thaw cycles (1, 3, 5) also had no obvious effect on the response of freezing treated cells to UV. The most noticeable reduction in the log inactivation level between the control (without freezing) and the freezing treated cells occurred at the lower UV fluence (6 mJ/cm²).

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