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Performance of coiled tube ultraviolet reactors to inactivate *Escherichia coli* W1485 and *Bacillus cereus* endospores in raw cow milk and commercially processed skimmed cow milk

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Abstract

Two coiled tube reactors were designed to investigate the influence of Reynolds number (R_e) and diameter of fluid carrying tube on UV-C inactivation of *Escherichia. coli* W1485 and *Bacillus cereus* endospores in raw cow milk (RCM) and skimmed cow milk (SCM) at room temperature. UV reactors were constructed using perfluoroalkoxy (PFA) tubing having internal diameters of 1.6 mm and 3.2 mm and each had a residence time of 11.3 s. Four levels of R_e were tested for each milk type, each tube size and each bacteria type. Inactivation efficiency increased as the R_e increased in both the reactors for both types of milk. The inactivation of both bacteria was higher in the 1.6 mm UV reactor than the 3.2 mm UV reactor. Maximum reduction of 7.8 $\log_{10}$ CFU/ml of *E. coli* was achieved in SCM in the 1.6 mm UV reactor corresponding to the R_e of 532 and higher, whereas the maximum reduction of *E.coli* in RCM was 4.1 $\log_{10}$ CFU/ml at the highest level of R_e (713) tested. For *B.cereus*, the maximum reduction was 2.72 $\log_{10}$ CFU/ml in 1.6 UV reactor, in SCM at R_e of 1024; whereas the maximum reduction of *B.cereus* in RCM was 2.65 $\log_{10}$ CFU/ml at R_e value of 713. Inactivation efficiency of both bacteria was more in SCM than RCM. The coiled tube reactor design provided adequate mixing and UV-C dosage for efficient disinfection of *E.coli* cells in milk.

Keywords: Ultraviolet C, ultraviolet reactor, nonthermal processing, cow milk, *E.coli*, *B. cereus*.

1. Introduction

Thermal processing of foods has been the most popular technology for food preservation. However, thermal processed foods were often reported to have cooked flavor, protein denaturation, loss of nutrients and vitamins (Wirjantoro and Lewis, 1997; Knorr, 1999). Moreover, reports suggest raw dairy products are preferred by consumers due to superior organoleptic characteristics (Buchin et al., 1998). Therefore there is an emphasis on developing nonthermal processing technologies with the goal of improving retention of quality and nutrition of food products (Knorr, 1999; IFT, 1999). Ultraviolet (UV) irradiation is one nonthermal technology that has garnered considerable interest for treatment of food.

Within the UV range, there are three regions based on the wave lengths in the electromagnetic spectrum: UV-A (315 - 400nm), UV-B (280 - 315nm) and UV-C (200 - 280nm). Studies (Bank et al., 1990; Miller et al., 1999) suggest the destruction of microorganisms occurs by penetration of UV-C light through the cell membrane resulting in damage of DNA due to the formation of thymine dimers which prevent DNA transcription and replication, eventually leading to cell death. The 253.7 nm in the UV-C region was reported to have the most efficient bactericidal effect because the DNA of microorganisms absorbs photons strongly at this wavelength (Harm, 1980).

When UV is used to treat food, the UV-C dose is the radiant UV-C energy incident on a unit surface area of food being treated. UV-C dose is normally calculated using the following equation (Quintero-Ramos et al., 2004).

$$\text{UV-C dose (mJ/cm}^2\text{)} = \text{Irradiance intensity (mW/cm}^2\text{)} \times \text{Exposure time (s)} \quad (1)$$

Where, Irradiance intensity is the incident intensity of UV-C light on the surface being treated. It may be either directly measured using a UV-C sensor or estimated if the intensity of the source and optical properties of transmission medium in known.

Different types of ultraviolet reactors for liquid food pasteurization have been reported in literature. Wright et al. (2000) used a commercial thin film UV reactor (CIDER-10uv) to inactivate *Escherichia coli* O157:H7 in apple cider by 3.8 $\log_{10}$ CFU/ml with a UV dose of 9.4 to 61.0 mJ/cm^2. Using another brand of a thin film UV reactor (CiderSure3500A), Hanes et al. (2002) reported the reduction of *Cryptosporidium parvum* oocysts by 6 $\log_{10}$ CFU/ml in apple cider at the UV dose of 14.32 mJ/cm^2. The earliest use of a coiled tube UV reactor for food treatment was reported by Geveke (2005) to reduce bacterial loads in apple cider. The author reported a reduction of *E.coli* K12 by 3.4 $\log_{10}$ CFU/ml. Using another coiled tube UV reactor, Koutchma et al. (2007) studied inactivation of *E. coli* K12 in pineapple, guava, apples, and lilikoi juices and reported 5-$\log_{10}$ CFU/ml reductions of *E. coli* K-12 in juices with absorption coefficient less than 15 cm^{-1}. Franz et al. (2009) also used a coiled tube UV reactor (UVivatec) and reported inactivation of *E. coli* and *Lactobacillus brevis* in cloudy apple juice to below detectable limits from an inoculum level of 10^6 CFU/ml or 10^4 CFU/ml.

A few studies have reported using UV-C to ensure microbial safety of goat milk and raw cow milk (RCM) of different fat percentages (Matak, 2004; Matak et al., 2005; Reinemann et al., 2006). With goat milk, greater than 5-$\log_{10}$ CFU/ml reduction of *Listeria monocytogenes* was achieved at a cumulative UV-C dose of 15.8 ± 1.6 mJ/cm^2 under turbulent flow conditions for a cumulative exposure time of 18 sec in a thin film UV reactor (Cider Sure 3500) (Matak et al., 2005). Using the same machine, Matak (2004) found efficacy of UV-C on inactivation of *E. coli*

ATCC 25922 (an *E. coli* O157:H7 surrogate) was highest with skimmed cow milk (SCM) (2.27 logs) followed by reduced fat milk (1.82 logs) and whole milk (1.44 logs) at different temperatures under laminar flow conditions. Matak (2004) reported the reduction of the *E. coli* O157:H7 surrogate in whole milk treated with UV-C at 4°C was significantly lower than milk treated at 20°C. Employing a turbulent flow UV reactor, UV-C was also reported to inactivate background flora present in the raw cow milk by 2.29 $\log_{10}$ CFU/ml of SPC, 2.55 $\log_{10}$ CFU/ml of psychrotrophs, and 1.67 $\log_{10}$ CFU/ml of thermodurics (Reinemann et al., 2006).

Flow regime plays a role in killing microorganisms using continuous flow UV reactors for treating liquid foods (Koutchma et al., 2007; Matak, 2004; Matak et al., 2005). Reynolds number (R_e) is the ratio of inertial forces to viscous forces and is expressed as:

$$R_e = (\rho/\mu) \times vD \quad (2)$$

where, R_e is Reynolds number, ρ is density of fluid, μ is dynamic viscosity of fluid, D is diameter of tube carrying the fluid, and v is velocity of flow (Geankoplis, 1993).

Laminar flow occurs when $R_e < 2100$, whereas $R_e > 4000$ indicates turbulent flow. A flow regime with R_e between these numbers is considered transient flow (Geankoplis, 1993). The flow pattern of a fluid in a coiled tube is known as Dean flow (Dean 1927). A secondary flow field accompanies laminar flow of fluid as centrifugal forces act on the fluid within the coiled tube. The Dean number D_e is the similarity parameter governing the fluid motion in coiled tube flow configuration.

$$D_e = R_e \sqrt{(D/D_c)} \quad (3)$$

where D is the tube diameter, D_c is the coil diameter, and R_e is the tube Reynolds number (Dean 1927). According to Dean's (1927) theory, the secondary flow vortices occur in the range of $0.03 < D/D_c < 0.10$. Koutchma et al. (2004) suggested that flow pattern and design of the UV reactor are critical factors for treatment of any liquid with UV-C. In a coiled tube UV reactor with Dean flow conditions, secondary flow vortices provide uniform mixing conditions of fluid particles, improving UV-C exposure (Koutchma et al., 2007; Franz et al., 2009).

There were outbreaks of *E. coli* O157:H7 in California, Washington, and Ohio due to consumption of RCM in 2006 (Int. Soc. for Infectious Diseases, 2006). In the study reported here, *E. coli* W 1485 was used as a surrogate of *E. coli* O157:H7 as a safety precaution. Bachmann (1972) reported that *E. coli* W1485 is related to *E. coli* K-12 strains. The strain is reported to be more resistant to UV-C than the pathogenic strain O157:H7 (Murakami et al., 2005). Thus, treatments that inactivate strain W1485 would be expected to be more effective with the pathogen.

Endospores produced by some Gram positive may survive pasteurization and cause processing problems for the dairy industry. Survival of endospores is a limiting factor for the shelf life of pasteurized milk. For example, *Bacillus cereus* is a potential food poisoning agent that can survive pasteurization and limit the shelf life of the fluid milk products (Magnusson et al., 2007).

Bandla (2010) evaluated *E. coli* W1485 inactivation in inoculated milk with a coiled tube UV reactor at different flow rates to determine a minimum residence time for a 5-log10 CFU/ml reduction. The flow rates ranged from 25 ml/min to 100 ml/min. The *E. coli* was inactivated by more than 8.5 $\log_{10}$ CFU/ml at flow rates up to 75 ml/min, corresponding with a minimum residence time of 11.3 s.

The objectives of this study was to design two coiled tube UV reactors for experimental purposes and examine their efficiency on inactivation of *E. coli* W 1485 and *B. cereus* spores in RCM and SCM. The effects of Reynolds number (R_e) and tube diameter with a constant

residence time of the milk product inside the UV reactor were investigated. A design consideration was for the two UV reactors to be identical except for tube size and tube length, and for the UV reactor with the smaller diameter tube to have Dean flow and the other to be as near Dean flow as possible.

2. Materials and Methods

2.1. UV reactors

Two UV reactors were designed for this study (Figure 1). The UV-C source for each was a 8.7 W, 110 V, UV-C germicidal lamp with peak emission at 253.7 nm, having a 505 mm arc length and 15 mm outside diameter (OD) (SBL325, American Ultraviolet Company, Lebanon, IN, USA). The UV lamp was enclosed within a quartz glass sleeve (American Ultraviolet Company, Lebanon, IN, USA) with a 22 mm OD and an air gap of 2.4 mm between UV lamp and sleeve. Perfluoroalkoxy polymer resin (PFA) tubing was selected to wrap around the UV reactor based on Geveke's report (Geveke, 2008) that PFA tubing is highly transparent to UV light and has more chemical and heat resistance than polytetrafluoroethylene and fluorinated ethylene propylene. One UV reactor was designed using 1.6 mm inside diameter (ID) by 3.2 mm OD PFA tubing, hereafter called the 1.6 UV reactor. Another UV reactor was designed using 3.2 mm ID by 4.8 mm OD PFA tube, hereafter called the 3.2 UV reactor. PFA tubing was wrapped in the form of coils around the UV lamp sleeve. Both UV reactors were covered with aluminum foil to prevent exposure to the personnel of UV light.

A range of R_e was required for this study and was achieved by using different flow rates of milk through the UV reactors and calculating the resulting R_e, based on equation 2 (Table 1). The density (ρ) and viscosity (μ) of RCM and SCM milk was estimated according to the relationship developed by Bakshi and Smith (1984). RCM had a lower density ($\rho = 1021.46$ kg/m^3) and higher dynamic viscosity ($\mu = 1.941\times10^{-3}$ Ns/m^2) than SCM ($\rho = 1024.34$ kg/m^3 and $\mu = 1.314\times10^{-3}$ Ns/m^2). This resulted in a higher kinematic viscosity (μ/ρ) in RCM (1.9×10^{-6} m^2/s) than SCM (1.27×10^{-6} m^2/s) and lower R_e for RCM.

In order to maintain a consistent residence time of product exposure to UV, different lengths of tubing were used for each flow rate (and corresponding R_e). A residence time of 11.3 s was selected based on the work of Bandla (2010).

The PFA tubing wrapped around the quartz glass sleeve was divided into two zones in both reactors (Figure 1). For the 1.6 UV reactor, the length of the tubing wrapped in the first zone was 240 cm and the second zone was 480 cm. For the 3.2 UV reactor, the length of the first zone of the tubing was 120 cm and the second zone was 240 cm. A three-way valve connected the two divided zones in each UV reactor, allowing flow through each zone only or through both zones. Four levels of R_e were achieved by changing the flow rate of milk in each reactor while keeping the residence time constant by allowing fluid flow through the first zone only, second zone only, first and second zone, and first and second zone with recirculation through the first zone. The recirculation was provided in a two step process. First, milk was pumped through the zone 1+2 and milk was collected, and then the collected milk was pumped through the zone 1 only. See Table 1 for a listing of length of PFA tubing for each R_e and flow rate. Using this arrangement, residence time of milk in the both reactors was the same at all levels of R_e. Flow rate was doubled for the 3.2 UV reactor compared to the 1.6 UV reactor at each R_e level to achieve the same R_e at each level in both UV reactors.

The UV-C dose was calculated using equation (1) by multiplying the residence time (11.3 s) with the irradiance intensity at the milk surface. The irradiance intensity was estimated by multiplying the UV-C intensity of the lamp at 5 mm from the lamp (1.375 mW/cm^2) with the transmittance of quartz glass sleeve (90%) and PFA tube (80% in germicide range). Using these values, the estimated UV-C dose for the designed reactors was 11.187 mJ/cm^2.

2.2. Calibration of flow rate in UV reactor

Prior to the experiments, the flow rates of RCM and SCM through the UV reactors were calibrated at 25, 50, 75 and 100 ml/min with the 1.6 UV reactor; and 50, 100, 150 and 200 ml/min with the 3.2 UV reactor. These flow rates produced different R_e levels (Table 1). During calibration, the inlet and outlet temperatures of milk were observed to be in the range of 23 - 24°C and 24 - 26°C, respectively. This temperature range was selected because the earlier studies reported a better kill rate at room temperature compared to that of the 4°C (Matak, 2004). Each type of milk (500 ml) was pumped through each UV reactor and collected in a graduated cylinder for 1 min with three replicates. The setting of peristaltic pump speed at each flow rate was recorded and a linear regression equation ($R^2 = 0.98$ for RCM and $R^2 = 0.94$ for SCM) was determined and used to set the pump speed setting.

During actual experiments, the pump speed dial was set per the respective regression equation. Actual flow rates were also measured during the experiments using a timer and a graduated cylinder. The difference between actual flow rates from the planned flow rates was ±1 ml/min at the set pump speed.

2.3. Milk collection

Fresh RCM was collected in an autoclaved glass bottle from the dairy farm of Southern Illinois University, Carbondale. Milk composition (fat, protein and total solids) was analyzed using an infrared analyzer (Infrared Analyzer, Denver Instrument Company, Arvado, CO) by the lab technicians of Prairie Farms (Carbondale, IL, 62901). Skimmed milk was purchased from a local grocery store. The milk samples were refrigerated until UV processing, about 5 - 6 h later.

2.4. Milk quality

Natural background floras were enumerated in RCM and SCM by standard plate count (SPC) and coliform count (Wehr and Frank, 2004). Dilution blanks were made up of phosphate-buffered saline (PBS). A series of dilutions were spread plated (0.1 ml) in duplicate onto tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI). Plates were incubated at 33°C for 24 - 48 hours. Natural presence of *E. coli* cells in RCM was assessed as per Marshall (2004) by using selective media namely, violet red bile agar (VRBA) with methylumbelliferyl-β-D-glucuronide (MUG) (Difco Laboratories, Detroit, MI).

2.5. Optical absorbance

UV-C absorbance of RCM and SCM was measured at 24 - 25°C with a UV-visible spectrophotometer (UV-1601, Shimadzu, Columbia, MD) at 254 nm wavelength by diluting each milk sample to 99% with deionized water. Since milk samples were not very transparent to UV-C, it was not possible to get absorbance reading. Therefore the samples were diluted and the resultant absorption coefficient values were multiplied by 100 to obtain estimated absorption coefficient of undiluted SCM and RCM. Disposable polystyrene cuvettes (Fisher Scientific) with a path length of 10 mm were used to measure the absorbance. The absorption coefficient was

determined as the ratio of absorbance of milk to path length of the cuvette used to measure the absorbance.

2.6. pH test

A pH meter (Corning, NY, USA) was calibrated using buffer solutions before measuring pH in both types of milk. With milk temperature at 24 - 25°C, pH was measured three times for each type of milk.

2.7. *E. coli* W1485 culture preparation

Stock cultures of *E. coli* W1485 were obtained from Dr. David P. Clark, Department of Microbiology, Southern Illinois University, Carbondale, IL. A colony was picked with a sterilized wire loop from the stock culture. This colony was inoculated in 120 ml tryptic soy broth (TSB) and incubated in a gyratory water bath shaker (New Brunswick Scientific Edison, NJ, USA) at 34°C for 18 - 24 hours (Marshall 2004). Serial dilutions were made and 0.1 ml of each dilution was spread plated onto TSA plates for estimating the cells present in the 120 ml TSB after incubation. These plates were incubated at 33°C for 18 - 24 hours and enumeration was determined by counting the colony forming units (CFU). An agar plate containing media without spread plating was also incubated at the same incubation temperature to assess if the poured media in petri plates was contaminated. The culture obtained from TSB was centrifuged (Beckman J2-M1, Schaumburg, IL, USA) at 12,000 rpm for 15 min at 4°C and the supernatant was discarded (Krishnamurthy et al. 2007). The pellet was immediately mixed with milk samples as described in section **2.9**.

2.8. *B. cereus* endospore preparation

B. cereus (ATCC Preceptrol® strain) was obtained from a -70°C glycerol stock supplied by the Department of Microbiology, Southern Illinois University, Carbondale, IL. Endospores of *B. cereus* were prepared according to Beuchat et al. (1997). Briefly, a pure-culture was inoculated into nutrient broth (Difco) and incubated at 30°C. Three successive loop transfers at 24 h intervals were made before spreading 0.1 ml aliquots onto the sporulation medium (Nutrient agar, Difco) supplemented with 0.05 g of $MnSO_4$ per liter. The plates were incubated for 72 h at 30°C and the spores were harvested by depositing 5 ml of sterile distilled water on the surface of each plate and rubbing gently with a sterile bent glass rod. The washing procedure was repeated twice. The resultant wash suspensions were centrifuged at 3000 rpm for 20 min at 5°C (Beckman J2-M1, Schaumburg, IL, USA) and the supernatant liquid was discarded. The resulting pellet was suspended in 100 ml of sterile distilled water and centrifuged at 6,000 rpm for 10 min at 5°C. This procedure was repeated twice and the final pellet was suspended in sterile de-ionized water and 5 ml each were distributed in 15 ml sterile centrifuge tubes. The tubes were placed in a water bath at 80°C for 30 min to kill vegetative cells (Beuchat et al., 1997). After 30 min holding time at 80°C, the centrifuge tubes were stored in a cold room at 4°C to inhibit further growth. Gram stains of the endospore preparation were observed with a light microscope for the presence of vegetative cells. After making sure all the vegetative cells were destroyed by the heat treatment process, 5ml spores contained in centrifuge tubes were stored in a cold room at 4°C to inhibit the further growth of vegetative cells. These centrifuge tubes were used in the subsequent experiments.

2.9. Inoculation of *E. coli* W 1485 and *B. cereus* spores

Pyrex glass bottles (2000 ml) with a magnetic stirrer were autoclaved and filled with 1500 - 1600 ml of the refrigerated RCM and SCM in separate bottles. An *E. coli* W1485 pellet

obtained from a 120 ml culture was directly added to each RCM and SCM container. This inoculated milk served as the source of milk that was treated in the reactors. The milk was slowly stirred with a stirrer for 30 min to warm the milk to room temperature (23-24°C) prior to sampling for microbial enumeration and UV-C treatment. Samples for microbial counts were serially diluted and 0.1 ml was spread plated onto petri plates containing VRBA with MUG (Difco Laboratories, Detroit, MI). VRBA with MUG is a selective medium to estimate the *E. coli* cells present in milk (Marshall 2004). This medium can differentiate *E. coli* from other species of coliforms by observing pink-colored *E. coli* colonies under long-wavelength UV light (Marshall 2004).

Sterile glass containers (2000 ml) were separately filled with 1500 – 1600 ml of RCM and SCM and inoculated with 5 ml of a *B. cereus* endospore preparation (see 2.8 above). The containers were slowly stirred on a magnetic stirrer for 15 - 20 min to suspend endospores uniformly in each milk type and then used for enumeration and UV-C treatment as described for *E. coli*. *B. cereus* endospores in the RCM and SCM were estimated using Mannitol egg yolk polymyxin agar (MYPA) (Beuchat et al. 1997). Sterile egg yolk and polymyxin vials were used to prepare MYPA media according to the manufacturer's directions (Difco Laboratories, Detroit, MI) (Donovan, 1958). Enumeration of microorganisms in untreated and treated milk samples was performed using plate counts as described in sections 2.7 and 2.8.

2.10. UV reactor operation and cleaning

Inoculated milk samples were treated using the UV reactors. A milk temperature of 24 - 26°C was observed during each of the experiments. The UV lamp was turned on three minutes before pumping milk through the reactors. The pump speed of the peristaltic pump was set per the calibrated settings to provide proper flow rates for each R_e. The milk sample (500 ml) was pumped through the reactor until all 500ml was finished. In case of 4^{th} level of Re, all 500 ml milk was pumped through the reactor once more, this time through zone 1 only. Treated milk samples were collected in sterile bottles and immediately stored in a cold room (4°C). Treated milk samples (0.1 ml) were spread plated on agar plates and incubated at 32 - 35°C. The control samples were prepared by pumping the inoculated milk samples through the reactors while the UV lamp was turned off. The same counting procedure used for the controls was applied to the treated milk.

Both UV reactors were cleaned immediately after each treatment by pumping hot water (500 ml) at 70°C followed by 100 ml hypochlorite recirculation (200 ppm) for 10 min, followed by 100 ml ethyl alcohol (95%) recirculation for 4 min and a final rinse with sterile 500 ml deionized water at room temperature. The final rinse water was collected in a sterile test tube and spread plated directly onto TSA plates to examine the efficacy of the cleaning procedure. After cleaning the UV reactors with the above procedure, there were fewer survivors than the limit of detection (10 CFU/ml) in either reactor.

2.11. Experimental design and statistical analysis

Factorial experimental design was used for this study. UV reactor (tubing diameter) and R_e were the main effects and log reduction of the bacteria was the dependent variable. Each of the four combinations of milk type (RCM and SCM) and bacteria (*E.coli* and *B. cereus*) was a separate experiment. The UV reactor variable treatments were 1.6 UV reactor and 3.2 UV reactor. The four levels of R_e for each of RCM and SCM are listed in Table 1. Each experiment consisted of eight treatments (2 UV reactors by 4 R_e levels), which were completed in a randomized order and replicated three times.

The results of each experiment were analyzed separately with factorial ANOVA (α = 0.05) using proc GLM in SAS 9.2 software (SAS, 2008). If the interaction of the main effects was significant, the simple effects of R_e within each UV reactor and the simple effects of UV reactor within each Re were determined. The *F* value for the simple effect was computed per O'Rourke et al. (2005). Tukey's studentized range test was used to determine differences among means of significant effects.

3. Results and Discussion

3.1. UV Reactors

UV reactors were constructed with two sizes of tubing: 1.6 mm ID and 3.2 mm ID. The coil diameter of the 1.6 UV reactor and the 3.2 UV reactor were 26 mm and 28 mm, respectively. The calculated D/D_c (equation 3) was 0.06 for the 1.6 UV reactor and 0.11 for the 3.2 UV reactor. The D/D_c for the 3.2 UV reactor was beyond the range of Dean flow ($0.03< D/D_c <0.10$), but as close as possible while maintaining the same UV lamp and sleeve as the 1.6 UV reactor. D/D_c for the 1.6 UV reactor indicates secondary vortices were developed inside the UV reactor, which were expected to promote mixing and provided uniform processing conditions. Secondary vortices (Dean flow) were expected to be less in the 3.2 UV reactor.

3.2. Quality of milk samples

Average composition of RCM was 4 ± 0.1% fat and 12.8 ± 0.15% total solids during our experiments. SCM composition was 0.1% fat and 9.1% total solids. The average SPC of RCM was 1.1×10^4 CFU/ml, whereas the average SPC for SCM was 4.2×10^3 CFU/ml. Coliforms and *E. coli* were not detected in either type of milk. The pH of RCM and SCM at 24°C was 6.7 - 6.8 which was within the range of normal pH (6.6 - 6.8) for both types of milk. UV-C absorption coefficient of RCM at 254 nm wavelength was 220 cm^{-1} whereas that of SCM was 170 cm^{-1}. The absorption coefficients of both types of milk were higher than that of fruit juices (11 - 78 cm^{-1}) reported by Koutchma et al. (2007).

3.3. Bacteria counts in control samples

The cell count of control samples (inoculated samples pumped through the reactor without UV lamp turned on) of RCM with *E. coli* and *B. cereus* were 8.66 and 7.74 $\log_{10}$ CFU/ml respectively. The control samples of SCM inoculated with *E. coli* and *B. cereus* had cell counts of 7.78 and 7.25 $\log_{10}$ CFU/ml respectively. These counts indicate the potential maximum log reduction of bacteria in the treated milk.

3.4. General observations

Tables 2-3 illustrate the relationships among UV reactor, R_e and mean number of log reductions, for each experiment. In all four experiments, the 1.6 UV reactor had a greater mean log reduction in bacteria than the 3.2 UV reactor at the same level of R_e. The 1.6 UV reactor benefitted from the combination of the smaller diameter tubing which resulted in a thinner path length for the UV-C to penetrate and the Dean flow which caused secondary vortices and better mixing of the milk. With the UV reactor design limitation of using the same UV lamp and quartz glass sleeve, the 3.2 UV reactor with the near Dean flow was not able to overcome the disadvantage of the thicker path length for the UV-C to penetrate.

Of the four experiments, bacteria removal to a non-detectable level was with the 1.6 UV reactor at $R_e \geq 532$ treating SCM inoculated with *E. coli*. None of the other treatments achieved the minimum requirement of 5-$\log_{10}$ reduction (FDA, 2003). However, mean log reduction of

bacteria increased with R_e in each experiment, regardless of UV reactor, up to the limit of the number of bacteria present. Greater log reduction was also achieved in SCM than RCM for *E. coli* while reduction of *B. cereus* was nearly the same for both types of milk. Based on the results presented, increased R_e levels in both UV reactors resulted in increased log reduction of *E. coli* cells and *B. cereus*.

3.5. *E. coli* inactivation in RCM

Factorial ANOVA indicated significant interaction between the main effects of UV reactor and R_e ($F_{(3, 16)} = 41.27$, $p < 0.0001$) on log inactivation of *E. coli* in RCM. The simple effect of R_e was significant for each of the 1.6 UV reactor ($F_{(3,8)} = 110.3$, $p < 0.0001$) and 3.2 UV reactor ($F_{(3,8)} = 20.32$, $p = 0.0004$). For the 1.6 UV reactor, log reduction significantly increased at each increase in R_e (Table 2). For the 3.2 UV reactor, log reduction of the two higher R_e levels was significantly higher than the log reduction of the two lower R_e levels.

3.6. *B. cereus* inactivation in RCM

Each of the main effects had a significant effect on log reduction of *B. cereus* spores in RCM. The 1.6 UV reactor had a significantly higher log reduction ($F_{(1,16)} = 19.04$, $p = 0.0005$) than the 3.2 UV reactor (Table 2). The effect of R_e, was also significant ($F_{(3, 16)} = 7.14$; $p = 0.0029$) on inactivation of *B. cereus* spores in RCM, with the lowest R_e of 181 having a significantly lower log reduction than the two higher R_e levels of 533 and 713. When R_e effects were isolated for each UV reactor, the highest R_e level had a significantly higher log reduction than the lowest R_e level for the 1.6 UV reactor ($F_{(3, 8)} = 5.2$; $p = 0.0277$) and there were no differences for the 3.2 UV reactor ($F_{(3, 8)} = 2.09$; *ns*) (Table 2).

3.7. *E. coli* inactivation in SCM

The interaction of main effects of UV reactor and R_e was significant ($F_{(3, 16)} = 80.3$, $p < 0.0001$) on log inactivation of *E. coli* in SCM. The simple effect of R_e was significant for each of the 1.6 UV reactor ($F_{(3,8)} = 496.4$, $p < 0.0001$) and 3.2 UV reactor ($F_{(3,8)} = 13.1$, $p = 0.0019$) (Table 3). For the 1.6 UV reactor, the treated SCM at three higher R_e levels had undetectable bacteria levels (highest possible reduction =7.78 $\log_{10}$ CFU/ml), which was significantly better than the log reduction of the lowest R_e level. This can be attributed to the significantly greater degree of mixing of SCM at higher R_e of 532 than the R_e of 265. Since the highest possible log reduction was already reached at this R_e level, the log reductions at R_e levels 794 and 1064 also had the same log reduction (7.78 $\log_{10}$ CFU/ml). For the 3.2 UV reactor, log reduction of the highest R_e levels was significantly higher than the log reduction of the two lowest R_e levels (Table 3).

3.8. *B. cereus* inactivation in SCM

The interaction of main effects UV reactor and R_e upon log inactivation of *B. cereus* endospores in SCM was significant ($F_{(3, 16)} = 8.69$, $p = 0.0012$) (Figure 5). The simple effect of R_e was significant for the 1.6 UV reactor ($F_{(3,8)} = 85.19$, $p < 0.0001$) with each higher level of R_e having a significantly greater log reduction, except for the two highest levels of R_e which were not significantly different (Table 3). Similarly, the simple effect of R_e was significant for the 3.2 UV reactor ($F_{(3,8)} = 4.31$, $p = 0.0436$), with the highest R_e level yielding a significantly higher log reduction than the lowest R_e level.

3.9. General Discussion

Inactivation efficiency of both bacteria increased as the R_e increased due to better mixing conditions inside the UV reactors. A similar inactivation pattern was not observed by Koutchma

et al. (2007) and Franz et al. (2009) because lower exposure time resulted from increased flow rates in their reactors while exposure time was kept constant at all levels of R_e in our study. Constant residence time with increased mixing likely accounted for differences in inactivation patterns when compared to the others studies. Therefore, we conclude that R_e number was the main factor responsible for the magnitude of inactivation of microorganisms by ultraviolet light.

Inactivation efficiency was higher for SCM than RCM for both bacteria. The inactivation differences between SCM and RCM in both UV reactors was due to the difference between UV absorption coefficients (170 cm^{-1} for SCM and 220 cm^{-1} for RCM) of both types of milk. Optical absorption coefficient at 254 nm of RCM was higher than SCM due to higher levels of suspended fat particles. This might have contributed to the greater reduction of both the bacteria in the SCM. Matak (2004) reported that *E. coli* was reduced by 1.44 logs at 20°C in whole milk at UV dose of 5.8 mJ/cm^2, residence time of 1.5 sec, and R_e of 1371 in a thin film reactor. The Dean flow within the 1.6 UV reactor yielded more reduction of *E. coli* at lower R_e compared to Matak (2004) in RCM. One may anticipate fouling of tubes in coiled tube reactors over time, reducing transparency of tubing and efficiency of microbial reduction. During this study we did not face this problem because we were cleaning after each treatment. In case of regular use, fouling may be avoided by following an appropriate cleaning schedule.

The initial temperatures of both types of milk were in the range of 23 - 24°C. The average increase in the final temperature of milk at the outlet of UV reactors was 4 - 6°C for 1.6 UV reactor and 2 - 3°C for 3.2 UV reactor. This mild increase in milk temperature should not have significantly affected inactivation of the test bacteria (Matak et al., 2005).

Throughout this study, inactivation of *B. cereus* endospores was lower than the *E. coli* W 1485 cells (Figures 2, 3, 4, and 5). *B. cereus* endospores are known to be highly heat resistant and can survive pasteurization whereas *E. coli* does not survive pasteurization (Marshall, 2004). Blatchley et al. (2005) found that UV inactivation kinetics of *B. cereus* endospores in aqueous suspensions (0.01M sodium bicarbonate) was characterized by a lag in inactivation for doses up to 15–20 mJ/cm^2, followed by roughly first-order inactivation. Inactivation response was roughly 4 log_{10} units at doses above approximately 30 mJ/cm^2, but tailed off at higher UV doses. In our study we could achieve maximum inactivation of *B. cereus* endospores of 2.71 logs, which was about 68% of the maximum achievable reduction (4 logs).

4. Conclusions

Inactivation of both the bacteria increased by increasing R_e at constant residence time in both UV reactors. The 1.6 UV reactor caused higher inactivation of *E. coli* W1485 and *B. cereus* endospores than the 3.2 UV reactor. The Dean flow condition and smaller tube diameter in the 1.6 UV reactor provided adequate mixing and UV exposure, as indicated by the lower UV dose for inactivation of *E. coli* W1485 cells in milk in this study than the studies on UV treatment of milk in larger coiled tube reactors or thin film reactors that were not Dean flow.

Inactivation efficiency of *E.coli* cells was higher for SCM than RCM with both the UV reactors. *B. cereus* endospores showed more resistant to UV dose than the *E.coli* W 1485 cells in both types of milk. *E. coli* W1485 in SCM was inactivated by more than target level of 5-log_{10} reductions whereas in RCM it was close (4.14 logs). Further, it may be possible to get FDA acceptable reduction ($\geq$ 5 log_{10} reductions) of *E. coli* in RCM by increasing the UV dose (residence time) in the 1.6 UV reactor. Higher thickness of milk requires better mixing

conditions. Therefore *E. coli* inactivation in 3.2 UV reactor may be achieved by increasing Reynolds number, residence time, or a redesign to provide Dean flow.

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Figure Captions

Figure 1. Diagram of coiled tube UV reactors designed for this study (drawing not to scale). For the 1.6 UV reactor, the inside diameter of fluid carrying PFA tube was 1.6 mm, whereas for the 3.2 UV reactor, it was 3.2 mm.

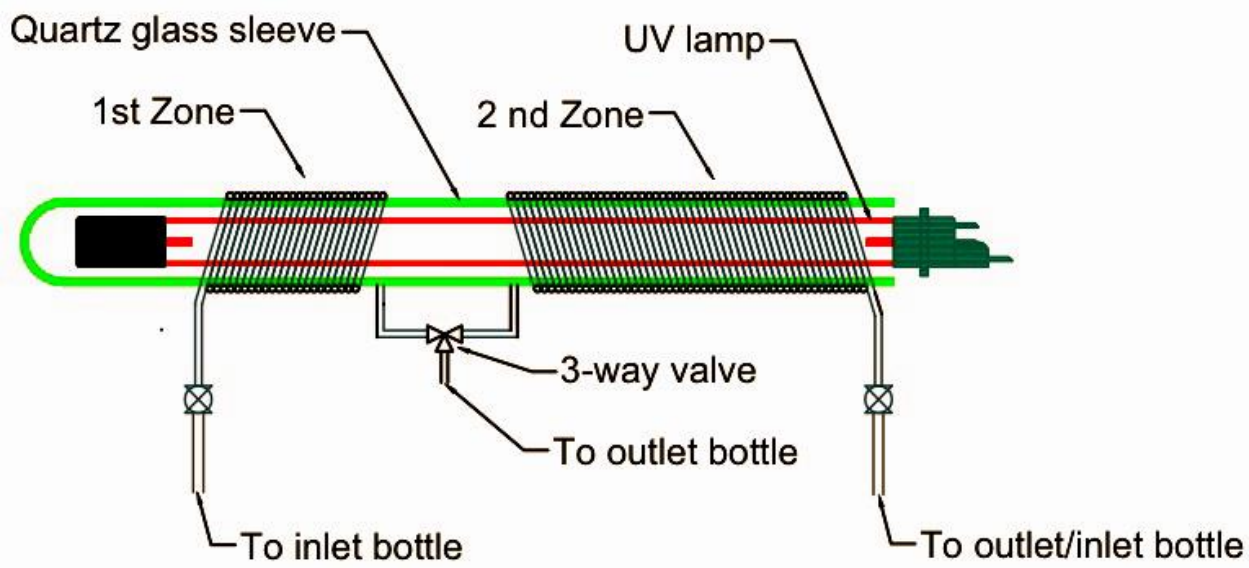

Figure 1. Diagram of coiled tube UV reactors designed for this study (drawing not to scale). For the 1.6 UV reactor, the inside diameter of fluid carrying PFA tube was 1.6 mm, whereas for the 3.2 UV reactor, it was 3.2 mm.

566 Table 1. Levels of flow rate, tubing length and Reynolds number (R_e) for each UV reactor.
567

	1.6 UV Reactor		3.2 UV Reactor		Mean R_e	
R_e Level	Flow Rate (ml/min)	Tubing Length (cm)	Flow Rate (ml/min)	Tubing Length (cm)	Raw Cow Milk (RCM)	Skimmed Cow Milk (SCM)
1	25	240	50	120	181	265
2	50	480	100	240	359	532
3	75	720	150	360	533	794
4	100	960	200	480	713	1064

Mean R_e values were calculated from observed flow rates during experiments. R_e values at each R_e level were same for both the reactors while they differed for each milk type due to the difference in kinematic viscosity of RCM and SCM. The residence time in each reactor was 11.3 s and accordingly the calculated UV doses provided were also fixed (11.187 mJ/cm^2).

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570 Table 2. Simple effects of R_e within each UV reactor upon inactivation of *E. coli* and *B. cereus*
571 endospores in RCM.
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	1.6 UV Reactor		3.2 UV Reactor	
Re	**E. coli***	**B. cereus***	**E. coli***	**B. cereus***
181	1.37[a]	1.28[a]	0.46[a]	1.06[a]
359	2.06[b]	1.96[ab]	0.63[a]	1.19[a]
533	2.95[c]	2.26[ab]	0.97[b]	1.38[a]
713	4.14[d]	2.65[b]	1.19[b]	1.72[a]

* Within each bacteria and UV reactor, R_e means with same letter are not significantly different

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575 Table 3. Simple effects of R_e within each UV reactor upon inactivation of *E. coli* and *B. cereus*
576 endospores in SCM.
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	1.6 UV Reactor		3.2 UV Reactor	
Re	**E. coli***	**B. cereus***	**E. coli***	**B. cereus***
265	2.06[a]	1.59[a]	0.47[a]	1.29[a]
532	7.78[b]	2.23[b]	1.12[a]	1.34[ab]
794	7.78[b]	2.57[c]	1.45[ab]	1.46[ab]
1064	7.78[b]	2.72[c]	2.43[b]	1.78[b]

* Within each bacteria and UV reactor, R_e means with same letter are not significantly different

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Nomenclature

B.cereus	*Bacillus cereus*
CFU	colony forming unit
CFU/ml	colony forming unit per milliliter
D	diameter (ID) of tube
Dc	diameter of tube coil
De	Dean number
E.coli	*Escherichia coli*
ID	inside diameter
μ	dynamic viscosity of fluid (milk)
mJ/cm^2	milli joule per square centimeter
ml/min	milliliter per minute
mW/cm^2	milli watt per square centimeter
MYPA	mannitol egg yolk polymyxin agar
nm	nanometer
OD	outside diameter
PFA	per-fuoro-alkoxy
ρ	density of fluid (milk)
RCM	raw cow milk
R_e	Reynolds number
SCM	skimmed cow milk
SPC	standard plate count
TSA	tryptic soy agar
TSB	tryptic soy broth
UV	ultraviolet
UV-C	ultraviolet C
v	velocity of fluid flow
V	volt
W	Watt