



Ultraviolet germicidal coil cleaning: Decreased surface microbial loading and resuspension of cell clusters



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ABSTRACT

Cooling coil surfaces within building ventilation systems are ideal sites for biofilm formation due to the presence of adequate nutrients (deposited particles) and moisture (condensate). In this study, a heating, ventilation, and air-conditioning (HVAC) test apparatus was built consisting of two parallel ducts, each with its own cooling coil. One coil was exposed to ultraviolet germicidal coil cleaning (UVG-CC) while the other was the comparison control to investigate the impact of UVG-CC on surface microbial loading and bacterial attachment. Surface samples were collected by swabbing a uniform area of coil surface and airborne samples were collected isokinetically with sterile funnel filters. All samples were quantified via direct epifluorescent microscopy. Prior to irradiating, higher concentrations of surface microbial loading were found on the downstream side of both cooling coils under condensing conditions. Conversely, under dry surface conditions with downstream UV irradiance, surface concentrations were higher upstream. UVG-CC (at an average 200 uW/cm^2 on the coil surface) reduced surface microbial loading by 55% on average during condensing conditions and inhibited bacterial attachment causing clusters of bacterial matter to become airborne downstream of the cooling coil. Additionally, it was found that desiccation also inhibited surface microbial loading and yielded cluster detachment but to a lesser degree than UVG-CC treatment.

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1. Introduction

Ultraviolet germicidal irradiation (UVGI) has a long history of being used for the disinfection of air streams, primarily in environments with higher risk of airborne pathogen transmission such as healthcare facilities, schools, and prisons [1]. UVGI systems use low-pressure mercury vapor lamps that emit shortwave ultraviolet-C, peaking at 253.7 nm. Using ultraviolet germicidal coil cleaning (UVG-CC) in heating, ventilation, and air-conditioning (HVAC) systems has recently gained popularity. While air disinfection may still occur as air passes by the UVG-CC system, the primary focus of UVG-CC is surface disinfection and, in turn, potential energy savings, maintenance cost savings, and increased or prolonged system capacity due to cleaner heat exchanger surfaces.

Heat exchanger surfaces are an ideal site for biofilm growth due to the presence of adequate nutrients (deposited particles and debris) and moisture [2]. High bacterial and fungal concentrations have been documented within HVAC systems, specifically on

cooling coil surfaces and drain pans [3–5]. Previous studies investigating the microbiological makeup of HVAC cooling coils observed the desiccation-resistant species of *Methylobacterium* to be the predominant organism recovered from the aluminum fins [6,7]. Other strains isolated from cooling coil biofilms include *Sphingomonas paucimobilis*, *Alcaligenes paradoxus*, *Bacillus cereus*, an unidentified *Sphingomonas*-like strain [6], and various members from the genera *Bacillus* and *Sphingomonas* [7]. In addition, *Methylobacterium* and *Sphingomonas* have been found to be predominant genera found in biofilms on household shower curtains [8]. The cyclical nature of these high moisture environments followed by extreme desiccation on both heat exchangers and shower curtains appears to be conducive to the formation of biofilms of *Methylobacterium* or similar bacterial members [7]. High concentrations of these and other organisms on heat exchangers have the potential to be resuspended into the airstream, possibly introducing allergens or toxins into the indoor environment.

Anecdotal evidence describes “visibly cleaner” cooling coils after the installation of a UVG-CC system but, to the authors’ knowledge, few peer-reviewed studies have investigated the fate of microbial contamination after irradiation. The objective of this

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study is to report detailed measurements of total microbial counts on surfaces and in air for an irradiated coil versus a non-irradiated coil under various operating conditions (i.e. dry coils versus condensing coils). Biological samples were analyzed via epifluorescent microscopy. Our hypothesis was that the UVG-CC system would keep the coil surface continuously clean of biofilm on the treatment side of the coil. The results presented in this study show that this hypothesis is only partially true, that the coil is cleaner than the control during the condensing operation mode of the system. These data allow us to provide recommendations for effective installation and operation of UVG-CC technology for mitigation of microbial contamination on HVAC heat exchangers.

1.1. Experimental methods

1.1.1. Test facility

An HVAC test apparatus was built in the Air Quality Laboratory at the University of Colorado Boulder, consisting of two parallel ducts (12-inch diameter), each with its own cooling coil (Trane, 1-ft² face area, 12 fins/inch, copper tubes and aluminum fins), but supplied by the same temperature and relative humidity controlled airstream (Fig. 1). The coils were steam cleaned prior to starting the tests.

One UVC lamp (ALTRU-V V-Ray Model 23-1100, 25W) was installed 10 inches away from the coil on the downstream side. The lamp was burned in for 100 h prior to use. The lamp was shielded with screen wire mesh to achieve the desired level of surface irradiance.

The test apparatus used indoor lab air from the room as the inlet air. The room HVAC system supplied 100% outdoor air filtered with MERV 14 filters. Air entered each cooling coil, on average, at 75 °F and 44% relative humidity and chilled water entered at 50 °F. The system mimicked a constant volume HVAC system, meaning the volumetric flow rate is maintained constant. The flow rates through each coil were kept equal to one another (350 CFM or 0.165 m³/s) using dampers since the static pressure drop across the coils may not be equal given equivalent flow rates. Air and water inlet temperatures, inlet relative humidity, and water flow rate were held as

constant as possible.

Fluctuations in outdoor air conditions affected conditions within the apparatus. During summer months, both coils had water actively condensing onto fin surfaces at nearly all times and drain pans were wet. In the winter months when outdoor air became very dry (wet bulb temperature ~57 °F), the apparatus was unable to humidify the air sufficiently to continue condensing water onto the cooling coils. These test periods of desiccation revealed interesting results, described in the Results section.

The system ran undisturbed for 4 months without UVG-CC on either coil to ensure that both coils fouled at an equivalent rate and to establish a robust baseline dataset. After 4 months of operation, the UV lamp was turned on, irradiating the downstream side of one of the cooling coils (called the *treatment* coil). The *control* coil was never irradiated. The irradiance at the surface of the treatment coil was on average 200 $\mu\text{W}/\text{cm}^2$, being roughly 280 $\mu\text{W}/\text{cm}^2$ at the center but 180 $\mu\text{W}/\text{cm}^2$ at the corners. Surface disinfection irradiance levels are much lower than what is typically used for air disinfection. Air disinfection applications may use irradiance levels upwards of 1000 $\mu\text{W}/\text{cm}^2$. Although irradiances on the order of 1 $\mu\text{W}/\text{cm}^2$ can be effective for surface UV applications, 50–100 $\mu\text{W}/\text{cm}^2$ is more typical per the Ultraviolet Air and Surface Treatment Chapter 60 in the ASHRAE HVAC Applications Handbook [9].

1.1.2. Sample collection

Coil surface samples were taken with sterile BBL CultureSwabs (BD, Sparks, MD). A 10-cm² area of the coil fin-edge surface was swabbed for each sample. Both the upstream and downstream side of each coil was swabbed. Swabbing was done at various locations, but the biomass was minimal at the corners, so we focused on sampling in four central quadrants (and found no difference in the results across quadrants). Swabs were aseptically cut and placed into 7 mL of HPCL water and vortexed for 1 min for extraction. Air samples were collected isokinetically with 0.45- μm cellulose nitrate membrane filters (Thermo Scientific, Waltham, MA) at 10 L/min for 19 h.

Air sampling was located downstream of where the duct air temperature was determined to be mostly uniform by collecting

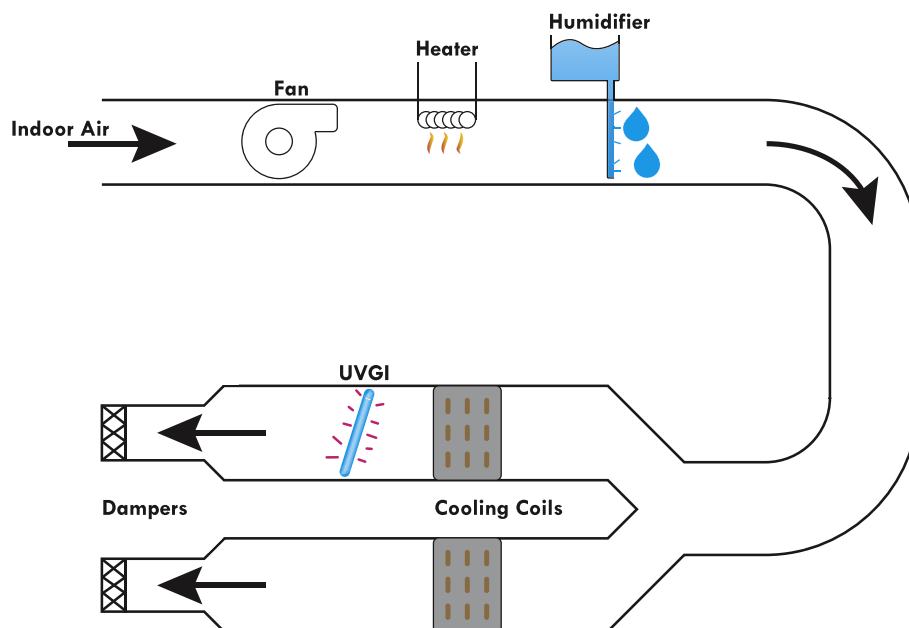


Fig. 1. Schematic of the cooling coil test apparatus.

temperature traverse data. Sampled filters were vortexed for 1 min in 7 mL of HPLC water for extraction. All samples were sieved through a 40- μm cell strainer (Fisherbrand, Pittsburgh, PA) to remove large particles and debris prior to staining. Samples were stained using SYTO BC Green Fluorescence stain (Life Technologies, Carlsbad, CA) and deposited onto a 0.2- μm black polycarbonate membrane (Millipore, Billerica, MA). SYTO BC is a nucleic acid stain that penetrates both Gram-negative and Gram-positive bacteria, yielding total cell counts (both live and dead).

No sample measurements were taken during January to June of 2015 since the coils were dry and nothing was changing on the coils, that is the biomass was low and stayed low, until the coils started condensing.

1.1.3. Enumeration by epifluorescent microscopy

Surface and air samples were directly counted using a fluorescence upright widefield microscope (Nikon E600) with a FITC filter. All reported counts are an average of 5 fields. In the case that the 5 fields from one sample yielded a coefficient of variance greater than 30% (non-uniform distribution on the membrane), the sample was discarded.

Total cell counts were used as a relative comparison between the UV-irradiated treatment coil and the control coil. Methods for sample preparation were consistent between all samples and we report results in average cell counts per field instead of estimated concentrations, which refers to the number of cells that were counted within the microscope field of view.

2. Results

Throughout one year of sampling, the cooling coils experienced three distinct modes of operation based on the conditions of the incoming air. These modes are:

- (1) *dry* – when air exiting the coil has not reached saturation and the coil surfaces are dry,
- (2) *transitional* – when air exiting the coil has reached saturation but water is not dripping into the drain pans, and
- (3) *condensing* – when air exiting the coil has reached saturation and water is actively condensing and dripping into the drain pans.

Surface microbial loading and resuspension of cell clusters was directly influenced by the mode of operation and the transition from one mode to another.

Prior to exposing one coil to UVG-CC, both coils were operating under mode (3), actively condensing water out of the air, for four months to establish a robust biofilm on the fin surfaces. Microbial concentrations changed over time on surfaces (Fig. 2) and in air (Fig. 3) both downstream and upstream for treatment and control coils. Figs. 2 and 3 present data from 15 discrete sampling days over the course of one year. Sampling occurred biweekly until dry conditions suspended our sampling due to no changes in microbial loading and very low counts from desiccation. Sampling was renewed in June of 2015 when condensing conditions returned. The first two sets of bars on the left side of Fig. 2 (labelled 8/26/14 and 9/9/14) represent the typical microbial loading profile for cooling coils in the summer months while actively condensing water. Due to large volumes of water present on fin surfaces, mostly located on the downstream side of the coil, microbial loading was highest on the downstream side (and in drain pan [3,10]).

The treatment coil began being irradiated on 9/19/14. Surface concentrations were reduced by 87% (standard error, SE = 4.6%) after one month of irradiation (from pre-treatment dates to 10/17/14). This effect, however, was confounded by the fact that both coils

were transitioning out of the actively condensing mode of operation. Desiccation caused downstream surface concentrations on the control coil to decrease by 55% (SE = 4.1%). Upon entering the summer months the following year and returning to condensing conditions, surface concentrations on the downstream side of the control coil began rising while concentrations on the treatment coil remained low. Counts were 55% (SE = 10%) lower on the treatment coil in the second condensing mode region (6/19/15 to 8/28/15). Over the entire year of sampling, downstream surface cell counts were 40% (SE = 13%) lower on the treatment coil than the control after the UV lamp was turned on.

Increases in airborne cell counts downstream of the coils coincided with reductions in surface counts (10/24/14 to 12/5/14), suggesting that as either UVG-CC or desiccation inactivates biofilms attached to the coil surface, clusters of cells detach and resuspend into the airstream (pictured in Fig. S1). Airborne counts downstream of the control coil were 57% (SE = 10%) lower than the treatment coil on average over all operational modes after UV was turned on.

During the condensing mode of operation, the surface conditions of the coil were favorable for microbial growth, depicted with a positive association between surface microbial loading and increasing surface wetness (or latent load), as seen in the left panels of Fig. 4. We suspect average surface counts on the treatment coil were inflated by the lag time for inactivation. The low level of irradiance resulted in long lengths of time to achieve inactivation dosage, possibly explaining the 2-week to one-month lag in decreased surface counts for the treatment coil during condensing modes of operation (Fig. 2). Conversely, there is a negative association between airborne counts downstream and surface wetness (right panels of Fig. 4). This suggests that biofilms are dependent on a certain amount of surface wetness to stay attached to coil surfaces or they are sloughed off of surfaces by the high air velocities through the fin channels in dry conditions.

Statistical t-tests were performed to determine if the sample means on every sampling date for the two sample types (surface and air), locations (upstream and downstream), and modes of operation (condensing, transitional, dry) were statistically different between treatment and control coils. F-tests were first performed to determine equality of variances and the appropriate t-tests was performed given that result. Table 1 only lists configurations that were statistically significantly different ($p < 0.01$) between control and treatment coils. Significant differences were primarily seen in downstream samples and were most common during the condensing mode of operation.

3. Discussion

SYTO BC Green Fluorescence stain is a mixture of SYTO dyes optimized for bacterial staining and counting. It is possible that this dye also stained fungi present in our samples, as bacterial viability kits using SYTO 9 have been successfully used for fungal spore viability assays [11]. The cooling coil environment that cycles through periods of heavy moisture and desiccation is an ideal site for fungi to thrive and numerous studies have documented fungal contamination in HVAC systems [4]. HVAC system fungal contamination may also play a role in the occurrence of sick building syndrome (SBS) [12,13]. Future studies of the efficacy of UVG-CC treatment for reducing cooling coil microbial contamination would benefit from the inclusion of fungal analyses.

The disadvantage of cell counts using a nucleic acid stain such as SYTO BC is that there is no differentiation between active and inactive cells. Methods exist for differentiating live and dead cells using microscopy techniques, one example being the LIVE/DEAD BacLight Bacterial Viability kit (Life Technologies, Carlsbad, CA). This

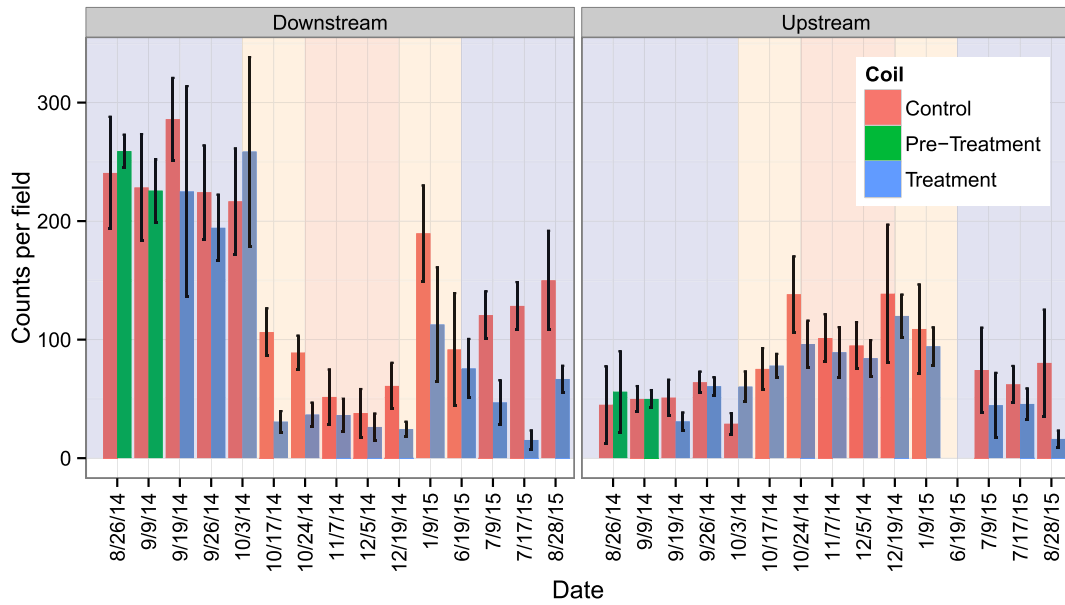


Fig. 2. Total cell counts per field of view from surface samples upstream and downstream of the treatment and control cooling coils by sampling date. The color of the bars represent which coil the sample was taken from and the color of the background represents the presence of moisture on the coil surface. Background areas shaded in blue correspond to condensing mode of operation, areas in orange correspond to transitional mode of operation, and the red shaded area corresponds to dry operating condition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

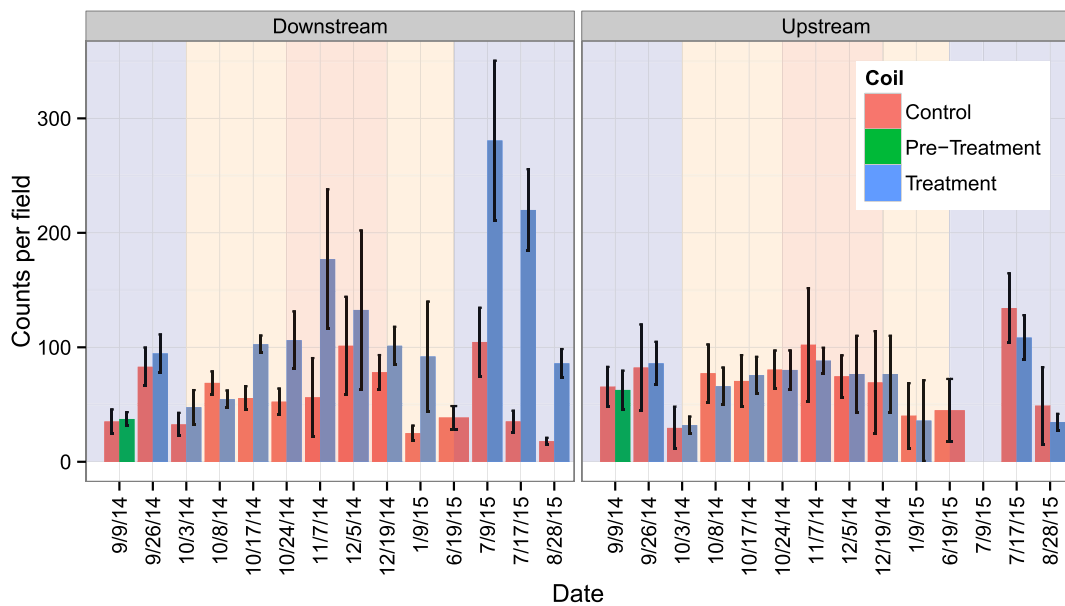


Fig. 3. Total cell counts per field of view from air samples upstream and downstream of the treatment and control cooling coils by sampling date. The color of the bars represent which coil the sample was taken from and the color of the background represents the presence of moisture on the coil surface. Background areas shaded in blue correspond to condensing mode of operation, areas in orange correspond to transitional mode of operation, and the red shaded area corresponds to dry operating condition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

kit consists of two stains, a green fluorescing SYTO 9 and red fluorescing propidium iodide (PI). SYTO 9 is able to enter any cell and stain the nucleic acid, often used for assessing total cell counts (as we did in this study). PI only enters a cell with a damaged cytoplasmic membrane to stain the nucleic acid red. The green stain is quenched in the presence of the red stain and energy is transferred to the PI stain if it is present in the cell. Low levels of UVC radiation inactivate cells by causing the formation of dimers in the DNA that prohibit replication. It is possible that an inactive cell with UV-induced DNA dimers may still stain as a live cell because its

cytoplasmic membrane is not damaged. Additionally, certain dyes (such as PI) readily adhere to particles and substrate material resulting in increased non-specific binding and background fluorescence, especially with fairly “dirty” environmental samples [14]. For these reasons we were unable to assess viability using microscopy techniques.

Culturing is another form of assessing bacterial viability. We attempted to culture both surface and air samples using tryptic soy agar and Reasoner’s 2 agar, as these were most successful in previous culturing studies of HVAC microbial contamination [3,7,15],

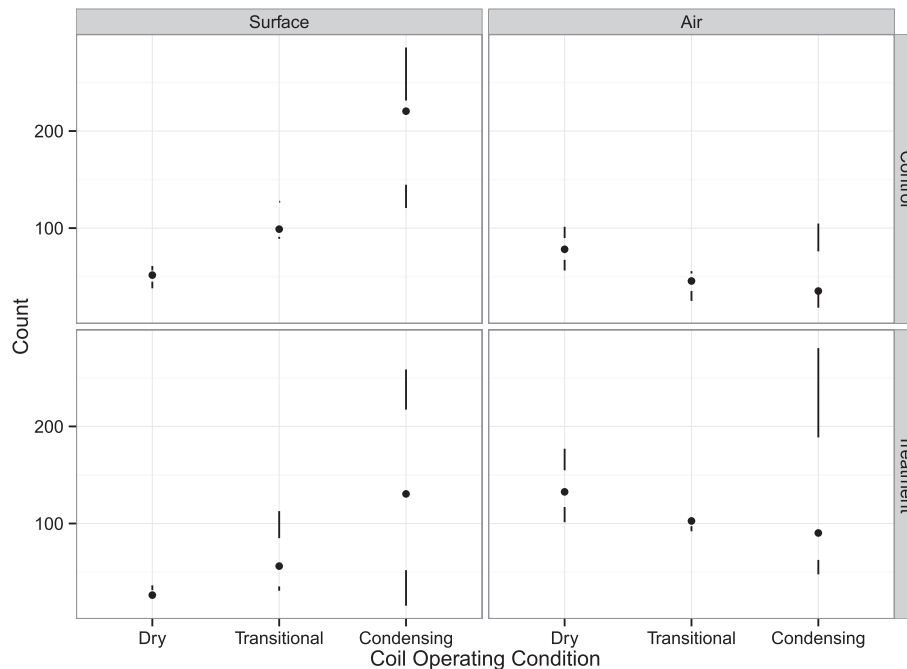


Fig. 4. Average microbial loading downstream for both coils is influenced by the coil operating conditions. When actively condensing, the presence of water promoted bioaerosol deposition and growth. When dry, microbial loading decreased on downstream surfaces and airborne concentrations increase as cell clusters are likely re-entrained into the airstream from the coil surface.

Table 1

Sample configurations with statistically unequal means between treatment and control cooling coils ($p < 0.01$).

Date	Sample type	Location	Mode of operation	p-value
9/19/14	Surface	Upstream	Condensing	$p = 0.007$
10/3/14	Surface	Upstream	Condensing	$p = 0.001$
10/17/14	Surface	Downstream	Transitional	$p = 2.6e-05$
10/17/14	Air	Downstream	Transitional	$p = 9.5e-05$
10/24/14	Surface	Downstream	Transitional	$p = 7.6e-05$
10/24/14	Air	Downstream	Transitional	$p = 0.002$
11/7/14	Air	Downstream	Dry	$p = 0.002$
12/19/14	Surface	Downstream	Dry	$p = 0.001$
7/9/15	Surface	Downstream	Condensing	$p = 0.0003$
7/9/15	Air	Downstream	Condensing	$p = 0.0006$
7/17/15	Surface	Downstream	Condensing	$p = 2.5e-05$
7/17/15	Air	Downstream	Condensing	$p = 7.9e-05$
8/28/15	Surface	Downstream	Condensing	$p = 0.0003$
8/28/15	Air	Downstream	Condensing	$p = 7.2e-05$

but were unable to consistently generate enough colony forming units for accurate counting. In many environments, up to 99% of microorganisms are unculturable [16]. This statistic combined with possible low airborne microbial concentrations from indoor air passing over the heat exchangers may have contributed to unsuccessful cultivation. Furthermore, our experimental setup had so little biomass that we were unable to use high-throughput sequencing techniques on our cooling coil surface samples due to unsuccessful DNA amplification using polymerase chain reaction (PCR).

The temperature and relative humidity of the air entering our test duct were mild compared to the condensing conditions of cooling coils in very hot, humid climates. We believe UVG-CC treatment is likely more effective in a region such as Southern Florida, with high cooling latent loads and possibly more robust and persistent biofilms, than a region such as Alaska with little to no cooling days annually [17]. Our laboratory setup is located between these two extremes, in Boulder, Colorado, with average

entering conditions of 75 °F and 44% RH compared to average August conditions of 85 °F and 72% RH in Miami, FL [18].

We were only able to investigate bacterial cell counts in this study, but it can be expected that fungal cells are also present in the coil biofilms. Fungal contamination of various HVAC system components, including air filters, insulation, and cooling coils, has been reported and fungal levels have been significantly lowered in air and on insulation within an air handling unit with the use of UVGI [4]. The potential of fungal resuspension due to UVGI may increase the risk of allergens or toxins being introduced into the air supplied to the building [19].

Unfortunately, we were unable to investigate whether the biofilm clusters sloughing off of the cooling coils were completely inactive or not. We also could not determine what taxa were present on the fin surfaces due to unsuccessful polymerase chain reaction (PCR) amplification caused by low biomass concentrations. Regardless of activity or classification, these microbes may still serve as potential allergens or toxins for the occupants in the building. Previous studies have identified Gram-negative bacteria on cooling coils [3,6,7] and higher concentrations of Gram-negative bacteria in indoor air have been correlated with buildings showing evidence of sick building syndrome symptoms [20]. This suggests that microbial matter released off of cooling coil surfaces has the potential to initiate adverse health effects.

Studies of wastewater have found that detached biofilm clusters in the presence of chlorine disinfection were able to survive and form new biofilms with relatively high viability, suggesting the possibility of perpetuating biofilm contamination due to reattachment and regrowth from the detached clusters [21]. It has also been shown that large bioclusters, particularly ones on surfaces, may shield and protect organisms within the core of the clusters from the harmful effects of UV irradiation [22]. For these reasons we recommend that UVG-CC installations consider using a higher level of surface irradiance than the recommended level for the first few months of operation in an attempt to inactivate detached microbes as much as possible. We also recommend that the final filter bank

after the cooling coil use at least a MERV 13 or higher during those first few months, or during periods of desiccation, when biofilm clusters will be sloughing off of the coil. After these “sloughing” periods, surface irradiance may return to the recommended level and final filters can be returned to the designed rating.

In summary, higher microbial loading occurred on downstream cooling coil surfaces in condensing conditions and, conversely, loading was higher on upstream surfaces in dry conditions. After initial fouling, reduction in surface loading coincided with increases in air concentrations downstream of the coils, suggesting resuspension of cell clusters from inactivated surface biofilms. Both UV irradiation and desiccation reduced microbial loading on surfaces and caused resuspension of cell clusters, desiccation to a lesser degree than UVG-CC by 57%. UV was most effective at reducing surface microbial loading in condensing conditions, with 51% lower surface concentrations downstream compared to the control in the second condensing mode region. In dry conditions, however, microbial concentrations on surfaces were statistically different on the UV-treated coil versus the control on just one sampling date. This work suggests that filters downstream of cooling coils in humid climates should be carefully monitored for proper installation and possible heavy loading after UV installation or coil desiccation, particularly after periods of high latent heat transfer loads that corresponds to large amounts of condensation on coil surfaces. We recommend replacing final filters one or two months after initial UVG-CC installation due to likely microbial fouling, especially if an increase in pressure drop across final filters is observed after the first few months.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.buildenv.2016.05.024>.

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