Overview of the University of Colorado Clean Air and Clean Water Studies

Accents In Water has just concluded studies in cooperation with Dr. Mark Hernandez, PhD, PE, Principal Investigator, to identify the impact that a water feature has on its environment. The studies were preformed at the University of Colorado at Boulder's Level 3 Lab in the Department of Civil, Environmental and Architectural Engineering.

Summary of Clean Air Delivery Rate (CADR) observations

This test was designed to define how effectively a water feature will reduce airborne particulate matter out of the air through its self generated electrostatic charge.

The results of this test are measured as a Clean Air Delivery Rate or CADR. The resulting CADR value is a measurement of how much air can be rendered free of bacteria and other microorganisms per hours of operation.

In separate experiments, Tuberculosis and Anthrax were introduced into the environment. Over a period of 5 hours, the number of bacteria in the air was reduced by at least 85%. The CADR for a 4x8 water feature was measured at 30 cubic meters per hour.

Summary of Modeling Analysis of UV inactivation

Why ultraviolet light? Ultraviolet sterilization systems require little maintenance and are a non-chemically based method of killing microorganisms. UV is a highly effective method of sterilization used throughout food processing, drinking water treatment and medical sterilization.

In this experiment, a different set of bacteria were chosen for their various levels of resistance to ultraviolet light. Independently tested were Legionella (Legionnaires Disease), Staph and Tuberculosis.

The objective of this study was to measure how effectively UV light is in deactivating, or killing a given microorganism. Each bacterium was independently tested against the UV to determine what percentage of bacteria survive passage through the UV chamber, thereby establishing a necessary dose of UV required to kill all bacteria.

Based on each of the testing models, the water is being dosed at levels far above what any of the bacteria are capable of surviving. We can safely say that Accents In Water can kill 99% of tested bacteria in a single pass through the UV chamber. Since our water features are recirculating systems, the water is constantly being cleaned, killing airborne microorganisms that are electrostatically attracted to the water feature.

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October 18, 2007

Via Electronic Mail
No Hardcopy to Follow

TO: David Black, Kloppenberg Inc.
FROM: Mark Hernandez, PhD, PE, Principal Investigator

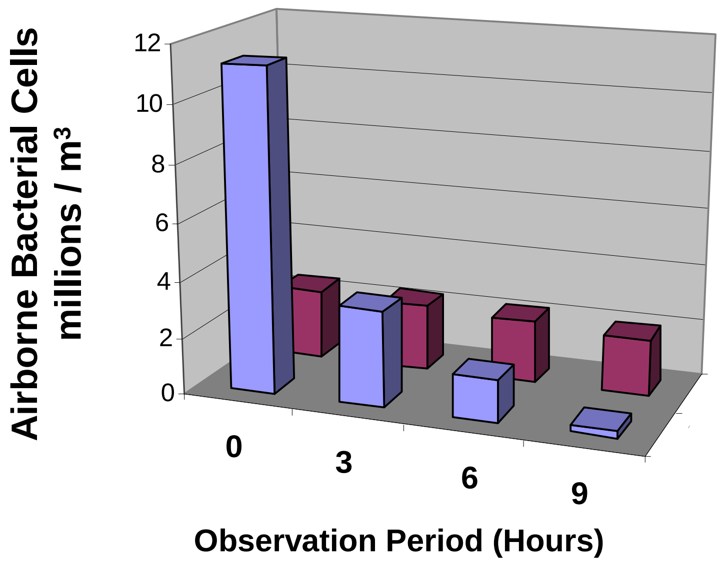
RE: RESULTS SUMMARY FOR MULTIPLE CLEAN AIR DELIVERY RATE (CADR) OBSERVATIONS OF ACCENTS IN WATER FEATURES CHALLENGED WITH MONODISPURSED PURE CULTURE BACTERIAL BIOAEROSOL UNDER DEFINED ENVIRONMENTAL CONDITIONS

OVERVIEW: Replicated experiments were performed by challenging three different ACCENTS IN WATER features, with known quantities of aerosolized *Mycobacterium parafortuitum* cells and *Bacillus subtilis* cells and spores. These cells were harvested fresh and healthy from laboratory broth culture in logarithmic growth phase. The challenge was executed at 30 ± 2 ^{o}C and 50 ± 5% RH.

Operational Challenge: We used *Mycobacterium parafortuitum* for these trials, because they are widely accepted as a surrogates for the environmental behavior and disinfection response of nosocomial diseases agents which cause Tuberculosis. *Bacillus subtilis* was also used for this study because this bacterium has been used as a surrogate for pathogenic bacteria in many bioaerosol studies, and the size response of of *M. parafortuitum,* and *B. subtilis* cells to relative humidity changes is negligible (Peccia, et al, 2001). Results from challenges with these Gram-positive cells are widely-accepted to conservatively represent remove response of resilient and pathogenic bioaerosols (exhaustive reference list available upon request).

EXPERIMENTAL DESIGN and FACILITIES: The University of Colorado has unique pilot (90m^3) bioaerosol facilities with the capability to support live bioaerosol challenges of full-scale disinfection equipment. This facility is designed to generate bioaerosols in conditions representative of many aerosol environments, but allow stringent control of environmental factors (temperature and humidity) that bioaerosols experience prior to, and during their transit through a test system. To achieve this control, we installed the ACCENTS WATER FEATURES within our full-scale laboratory chamber, and executed the tests defined by the operational configuration presented in the proposal (delivered summer 2007). One mode was a CADR test which characterizes the airborne microbe removal capability of these features in a recirculation mode representative of performance in a well-mixed room. The interpretation of the equivalent **C**lean **A**ir **D**elivery **R**ates (CADR) observed here can be extended to the operation of a water feature in an ideal environment: Where the CADR reported in units of volume/time (m^3/hour) is representative of how much air a given feature can render free of these bacteria in an hour.

RESULTS SUMMARY to DATE: Total airborne bacterial numbers were directly determined with sensitive biological stains in accordance with established microscopy methods (Hernandez et al, 1999) – For statistical purposes all DIRECT MICROSCOPIC AEROSOL COUNTS are taken as an average of 10 observations from at least three independent trials. **CLEAN AIR DELIVERY RATES** associated with the operation of each waterfall are summarized below for each of the different bacterial cultures tested.



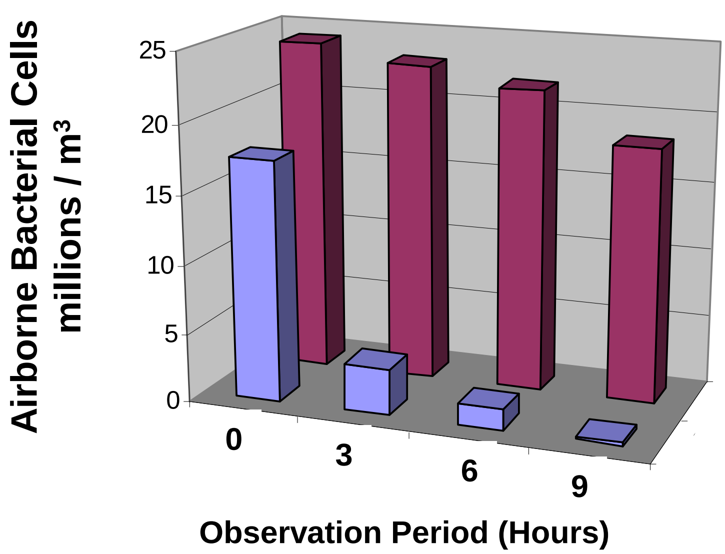
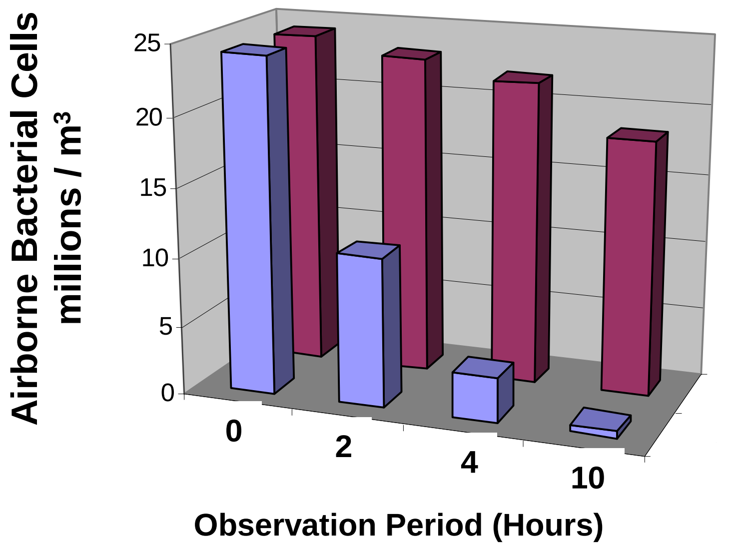


Figure 1. Room air concentration of *Bacillus subtilis* cells in during the operations (■), or disengagement (■) of a 2' x 3' stainless wire mesh ACCENTS water feature in a 90 m^3 bioaerosol chamber operated in a static, well mixed mode. In independent trials, room air was charged with the airborne bacteria

concentration noted at time zero, and monitored for 10 hours.



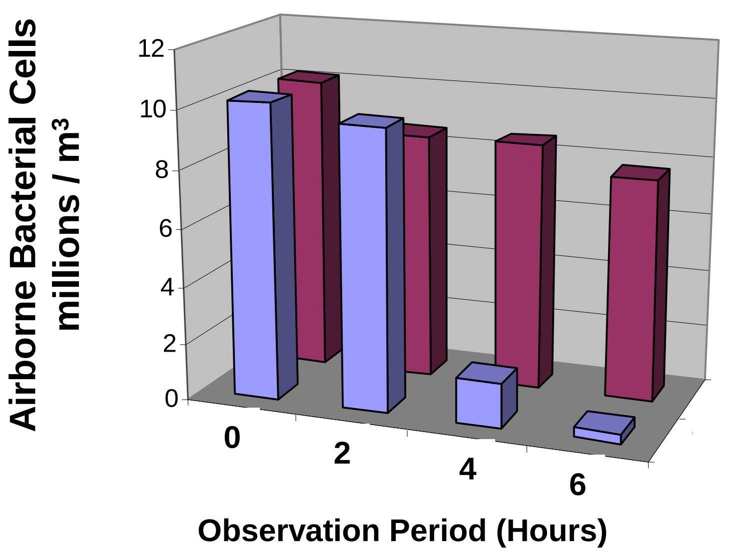


Figure 2. Room air concentration of *Bacillus subtilis* cells (top), and *Mycobacterium parafortuitum* cells (bottom) during the operations (■), or disengagement (■) of a 3' x 7' stainless wire mesh ACCENTS water feature in a 90 m^3 bioaerosol chamber operated in a static, well mixed mode. In independent trials, room air was charged with airborne bacteria concentration at the room air concentration noted at time zero, and monitored for between 6 and 10 hours.

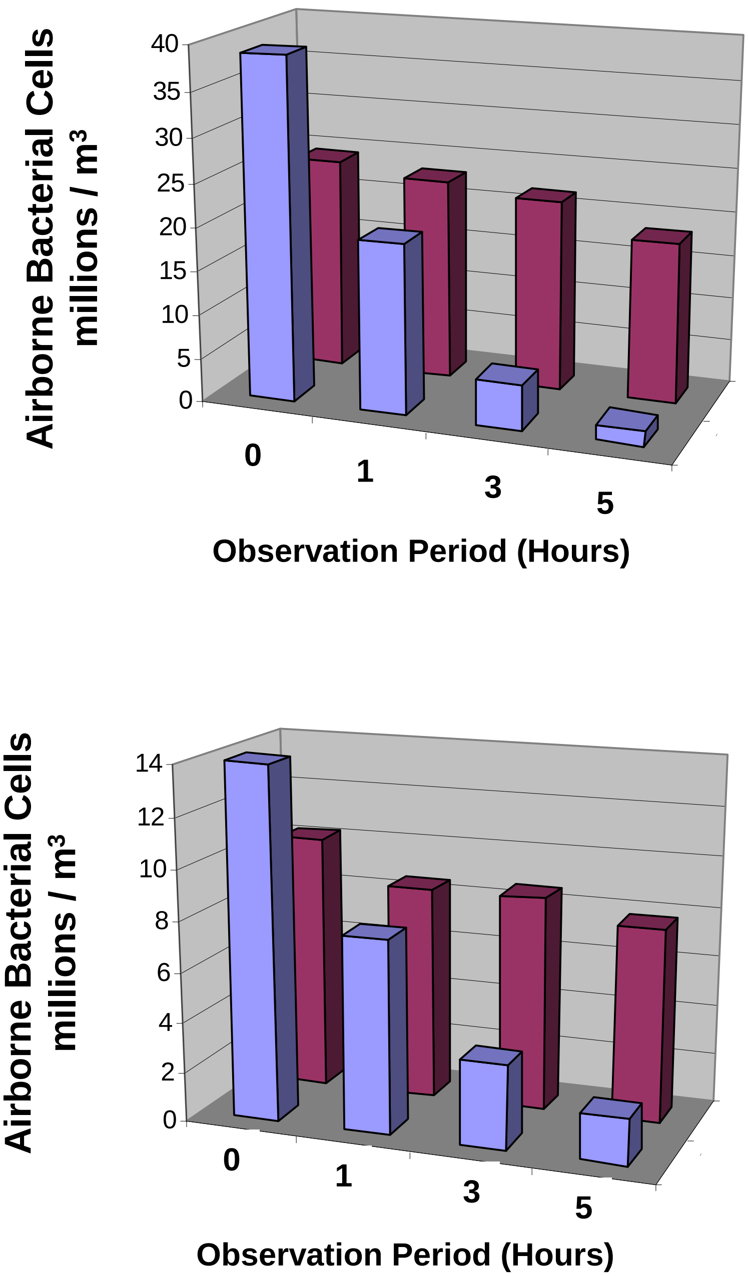
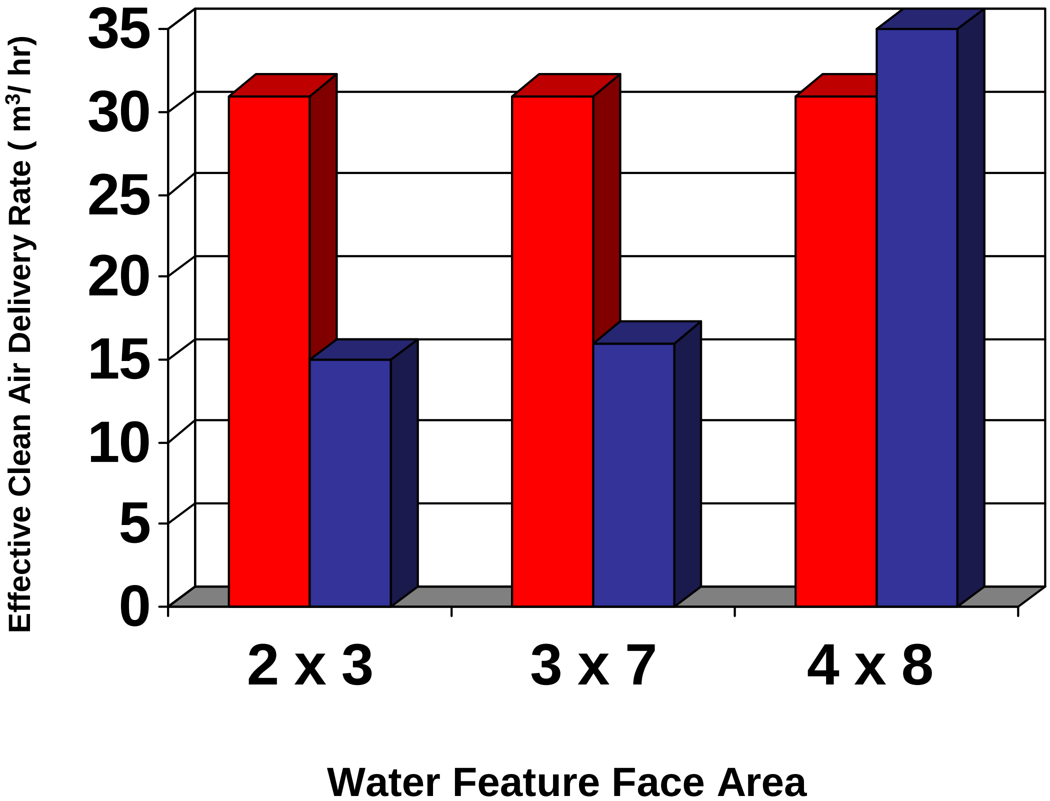


Figure 3. Room air concentration of *Bacillus subtilis* cells (top), and *Mycobacterium parafortuitum* cells (bottom) during the operations (■), or disengagement (■) of a 4' x 8' stainless wire mesh ACCENTS water feature in a 90 m^3 bioaerosol chamber operated in a static, well mixed mode. In independent trials, room air was charged with airborne bacteria concentration at the room air concentration noted at time zero, and monitored for between 6 and 10 hours.



Clean air delivery rate (CADR) observations were replicated and compiled for each of the unit sizes, using the protocols outlined above. DIRECT airborne bacteria counts dropped significantly when the bioaerosol chamber contained an operating water feature, regardless of size. These results suggest that on average, the effective CADR when challenged with airborne *Bacillus subtilis* cells (■) was between 15 m^3/hr and 30 m^3/hr, and approximately 30 m^3/hr when challenged with airborne *Mycobacterium parafortuitum* (■). These CADR averages apply when the water features contained clean distilled water, were operated at their maximum flow rate, and were equilibrated with the air in a 90m^3 room maintained at 30 C.

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TO: David Black, Kloppenberg Inc.
FROM: Mark Hernandez, PhD, PE, Principal Investigator

RE: Modeling Analysis of UV inactivation potential against selected pathogenic microorganisms retained by ACCENTS IN WATER features.

OVERVIEW: Isolated chamber experiments have shown that ACCENTS IN WATER features remove types of airborne bacteria that are often used as models for pathogenic bioaerosols (*Mycobacterium parafortuitum* and *Bacillus subtilis*). When challenged with high levels of airborne microbes, these water features removed these bioaerosols with an effective clean air delivery rate greater than 10 m^3/ hour. Once entrained in the water feature's flow, bacteria are recirculated through the feature's reservoir using a submersible pump which contains an in-line, low pressure ultraviolet lamp manufactured by Danner Inc., New York. Using the EPA Ultraviolet Disinfection Guidance Manual[1], (EPA 815 D 03 007), the inactivation potential of some airborne bacteria retained by ACCENTS IN WATER feature was assessed.

METHOD: The ultraviolet inactivation kinetics of a large number of pathogenic microorganisms that are significant to the microbial safety of water has been calculated from studies where UV exposure has been determined under optimal conditions: where the water is clean, has low turbidity, and high UV transmittance. The operation and maintenance of ACCENTS IN WATER features recommends pretreatments of water used to fill the feature's reservoir which qualifies this condition for UV inactivation analysis. The low pressure mercury lamps used in ACCENTS IN WATER features have been well characterized, and have approximately 85% of their power output as 254 nm monochromatic UV radiation. These lamps are housed downstream of a pump chamber which recirculates the feature's water past a UV lamp at a specified flow rate. We report here, estimates of bacterial inactivation potential when suspended in these feature's water through the range of flow rates recommended for normal operations. Widely accepted models[1,2] for UV inactivation were applied for these estimates using standard culture-based assays:

$$\text{Log}\ (N_t / N_o) = -k\ \text{Fluence}$$

Where culturable bacteria following the residence time of the unit (N_{ti}), with respect to their numbers just prior to exposure (N_o), are proportional to an inactivation rate k (mJ/cm^2), the UV power density and the time exposed. The product of UV power density and the time exposed is termed Fluence. The sensitivity of different microorganism to UV exposure is expressed by the inactivation rate k.

MODELED VALUES: The UV fluence was calculated for the geometry and mean *one pass* residence time of well-mixed UV chambers in ACCENTS in WATER features, and compared to accepted literature values for potential pathogens (*Mycobacterium avium, Legionella pneumophila*, and *Staphylococcus aureus*). These calculations use the following conservative assumptions: (i) where 85% of the nominal power is present as monochromatic UV (254 nm), and (ii) where 10% of the UV is lost to non-specific occlusion of the quartz sleeve; 10% is lost to bulb aging effects; and 20 % is lost to non-ideal water transmittance (reductions collectively denoted as aged). Using the conservative operations estimates outlined above, the UV doses delivered by *Pondmaster* units installed in the larger ACCENTS in WATER features are summarized below for the range of flow rates they deliver. They are presented with the corresponding UV doses considered in the best available literature for a range of potential pathogens.

1 Ultraviolet Disinfection Guidance Manual, United States Environmental Protection Agency 815-D-03-007
2 Hijnen, et al., Inactivation credit of UV radiation for viruses, bacteria and prozoan (oo)cysts in water: a review. Water Research (40) 2006

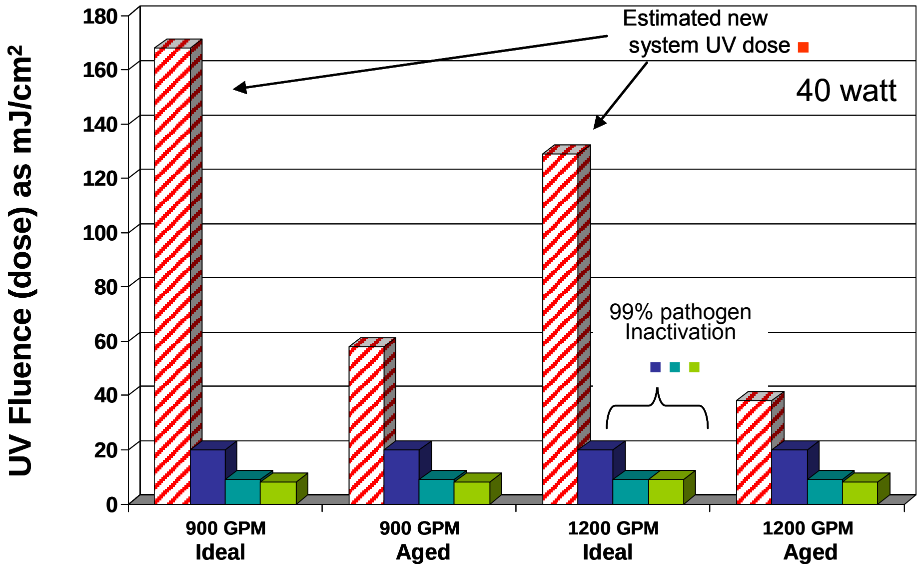

Figure 1. One pass UV dose delivered (■) in 40 W low pressure monochromatic chambers (c.a. 1100 cm^3) installed in ACCENTS in WATER FEATURES operated through the range of manufacturers recommended flow rates (900 GPM – 1200 GPM). Ideal estimate is for 85% of nominal fluence through clean water with no UV absorbance losses. Aged estimate incorporates composite of 40% UV absorbance losses due to sleeve occlusion, bulb losses, and depressed water transmittance. Exposure required for 99% inactivation of *Mycobacterium avium* (■), *Legionella pneumophila* (■), and *Staphylococcus aureus* (■) is presented for comparison.

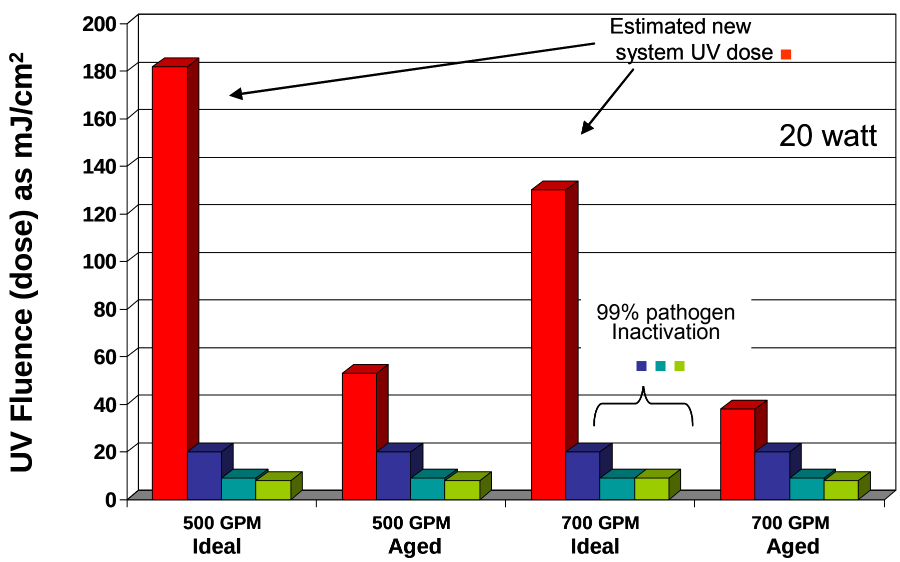

Figure 1. One pass UV dose delivered (■) in 20 W low pressure monochromatic chambers (c.a. 1100 cm^3) installed in ACCENTS in WATER FEATURES operated through the range of manufacturers recommended flow rates (900 GPM – 1200 GPM). Ideal estimate is for 85% of nominal fluence through clean water with no UV absorbance losses. Aged estimate incorporates composite of 40% UV absorbance losses due to sleeve occlusion, bulb losses, and depressed water transmittance. Exposure required for 99% inactivation of *Mycobacterium avium* (■), *Legionella pneumophila* (■), and *Staphylococcus aureus* (■) is presented for comparison.

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TO: Guy Bertelli, Accents in Water, Inc.
FROM: Mark Hernandez, PhD, PE, Principal Investigator

RE: UV inactivation response of microbial blooms in ACCENTS IN WATER features

OVERVIEW: Full-scale experiments have shown that ACCENTS IN WATER features remove airborne particulate matter that may serve as a substrate for bacteria in an operating water feature. Once entrained in the water feature's flow, bacteria and organic carbon can be recirculated through the feature's reservoir using a submersible pump which contains an in-line, low pressure ultraviolet lamp. Using the EPA Ultraviolet Disinfection Guidance Manual[1], the inactivation potential of different types of bacteria retained by ACCENTS IN WATER feature was previously assessed by modeling. We report here, the inactivation potential of this UV equipment against actual microbial blooms, which were purposely induced by operating 12' x 8' stainless steel water feature in a high-exposure environment with its UV disengaged, and with no SANIGARD pre-treatment.

METHOD: The ultraviolet inactivation kinetics of many different pathogenic microorganisms have been derived from studies under near optimal conditions: where water is clean, has low turbidity, and high UV transmittance. We purposely challenged a UV unit in an operating ACCENTS IN WATER feature under a conservative engineering condition where water quality conditions had degraded turbidity to levels greater than 5 NTU, total organic carbon (TOC) levels greater than 5 mg/L, and heterotrophic colony forming units exceeded 10^7 CFU/mL. These conditions were a purposeful result of 4 months of continuous operation during which the unit was not cleaned. We report here, the response of a bacterial enrichment when suspended in these feature's water through the maximum flow rate recommended for normal operation of the low pressure mercury lamps used in ACCENTS IN WATER features

INACTIVATION RESPONSE: Using the conservative operational scenario outlined above, the effects of UV doses delivered by *Pondmaster* units installed in the larger ACCENTS in WATER features are summarized below at the maximum flow rate they deliver.

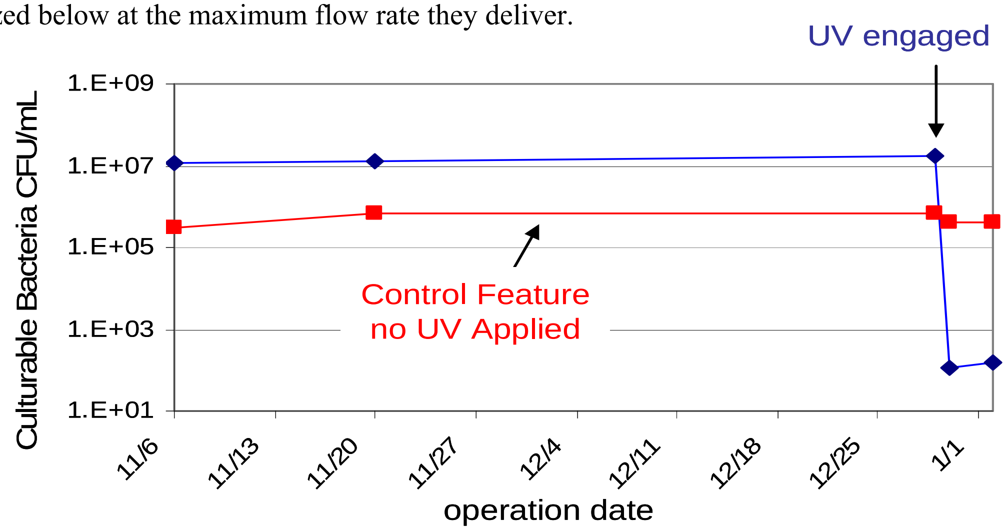

Figure 1. UV induced inactivation of bacteria enriched in an untreated 12 x 8 stainless water feature following 4 months of undisturbed operations (– ♦ –); and parallel unit operated under identical conditions during the same time period, but not subject to UV (–■ –).

RESULTS SUMMARY. The relatively high levels of culturable planktonic bacteria that were purposely allowed to build up in the water features were markedly impacted by the initiation of UV irradiation delivered by a Pondmaster plug flow unit. Even under the poor water quality conditions of the recirculating water (> 5 NTU turbidity), the concentrations of culturable microbes experienced a 5-log reduction in response to UV exposure, and did not experience any significant recovery on the order of days. These results are consistent with efforts to analytically model the inactivation of waterborne microbes under both clean and conservative conditions. While maintenance protocols are designed to prevent water quality condition from degrading to those observed here, these results suggest that the design of the UV irradiation system is effective and appropriate for the water features' configuration and the indoor environments in which they are intended to operate.

[1] Ultraviolet Disinfection Guidance Manual, United States Environmental Protection Agency 815-D-03-007

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TO: Guy Bertelli, Accents in Water, Inc.
FROM: Mark Hernandez, PhD, PE, Principal Investigator

RE: RESULTS SUMMARY FOR COMPARING SANIGARD EFFECTS ON ORGANIC CARBON SEQUESTRATION AND MICROBIAL COLONIZATION OF ACCENTS IN WATER FEATURES UNDER REALISTIC OPERATING CONDITIONS

OVERVIEW: Over a several month period, experiments were performed by monitoring the culturable bacteria content, as well as the organic carbon retained by 8' x 4' stainless steel ACCENTS IN WATER features that were otherwise identical except for the application of the antifouling agent ***SANIGARD***. The features were placed side-by-side in a relatively high pedestrian traffic area of the University of Colorado's Engineering Center, where they were operated continuously during the 2008 fall semester.

OPERATIONAL CHALLENGE: Organic carbon measurements, and standard heterotrophic plate counts commonly used to judge the quality of drinking water, were used as performance indices to isolate and judge the effect of a sole process variable: the pre-application of SANIGARD. We used heterotrophic plate counts for these trials because they are widely accepted as a surrogate indicator for the survival potential for pathogenic microorganism in drinking water. We also monitored both total and dissolved organic carbon content as indirect evidence of airborne particulate matter partitioning into the operating water features to link the following biophysical responses: (i) that the partitioning of particulate matter from the indoor atmosphere is a likely source of carbonaceous substrates for microbial growth in water features, and (ii) that SANIGARD applications may affect microbial growth in operationally significant time frames.

SETTING and FACILITIES: One of the busiest buildings on the University of Colorado's flagship campus is its engineering center, which is the academic home to thousands of students. The lobby of this facility was designed with and HVAC system providing a nominal air exchange rate between 2 and 3 air changes per hour (ACH), and has relatively high occupancy and pedestrian activity during normal business hours. This facility has relatively stringent control of environmental factors (temperature and humidity), for human comfort. We installed stainless ACCENTS WATER FEATURES each with an approximate surface area of 32 ft^2, side-by-side within this lobby, and monitored their operations continuously using a remote, internet-based camera.

RESULTS SUMMARY: Immediately prior to operations, the features were carefully cleaned with ethanol, and filled with sterile, particle free water. Airborne particulate matter from the indoor atmosphere partitioned into the water recirculating in the features; some of which contained organic carbon (TOC = Total Organic Carbon), and subsequently dissolved (DOC = Dissolved Organic Carbon).

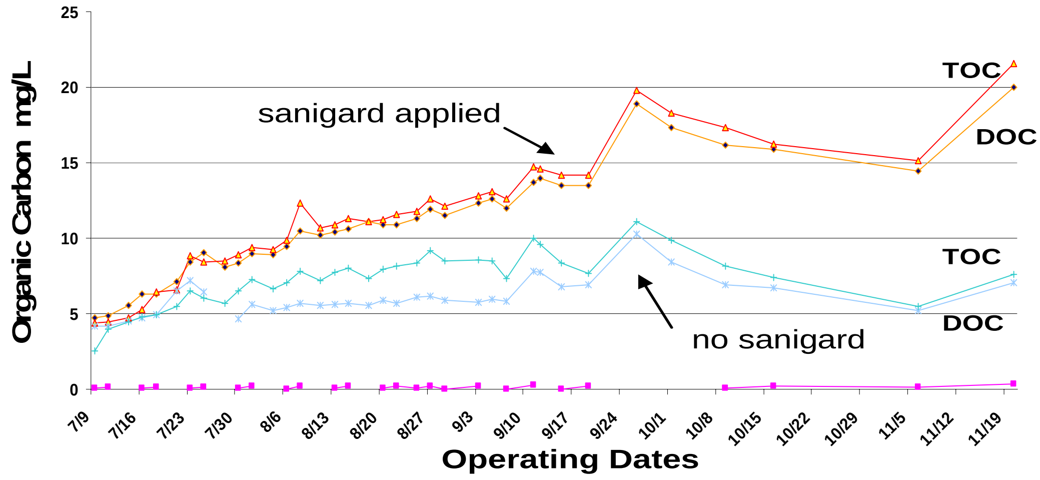

Figure 1. After more than 4 months of continuous operation, water features experienced marked increases in the amount of organic carbon sequestered from indoor air. In all cases, most of the organic carbon presented as a dissolved faction. Organic carbon was markedly higher in the water feature treated with the microbial anti-fouling compound SANIGARD (-▲-), than an otherwise identically constructed and operated unit not treated with SANIGARD (-▲-). Analytical TOC "blanks" are shown (-▲-).

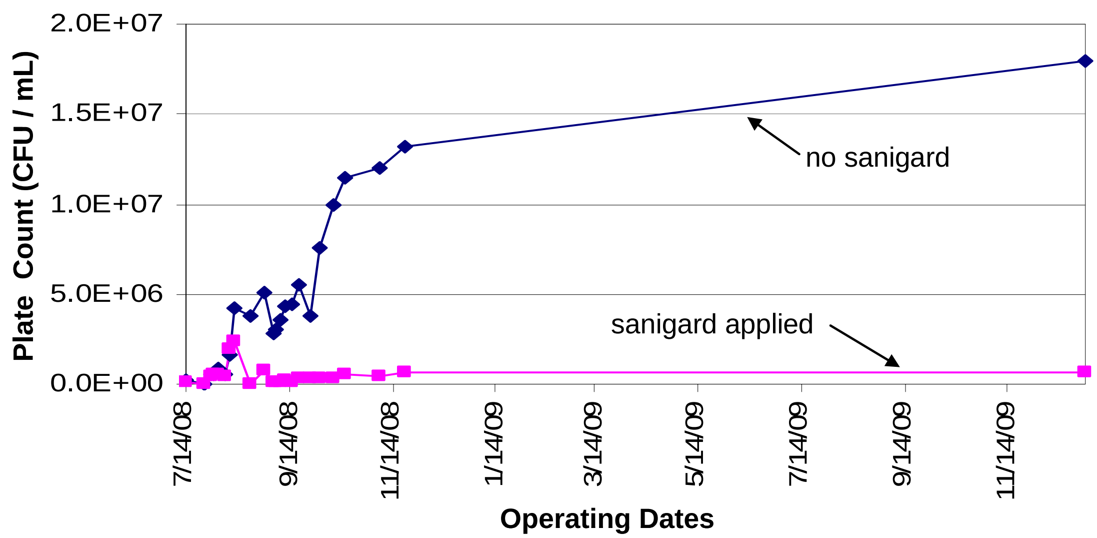

Figure 2. As judged by standard heterotrophic plate counts on broad spectrum bacterial media (R2A agar), the feature constructed of virgin stainless steel (-▲-)hosted more than 3 orders of magnitude higher heterotrophic colony forming units that an otherwise identical unit with steel surfaces covered with the antifouling compound SANIGARD (-▲-) during a 4 month period.

CONCLUSIONS and SIGNIFICANCE: These results suggest that the thin water films recirculating on the faces of these water features can sequester and retain airborne particulate matter from indoor environments with relatively high occupancy. This particulate matter has significant organic carbon content, which may be available as microbial substrates. This is consistent with increasing TOC/DOC content of the water feature reservoir in the absence of any clear indications of photosynthetic activity (chlorophyll A) or other significant autotrophic activity.

Given that the units were initiated with identical aliquots of sterilized, particle free drinking water, patterns of TOC/DOC increases and culturable heterotrophic microorganisms recovered from the water features' reservoirs, suggest that SANIGARD pretreatment had a significant inhibitory effect on the growth potential of attached and planktonic microorganisms. This performance is consistent in isolating this process variable, where the water features were identical in every engineering respect except for SANIGARD application.