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TECHNICAL PAPER

Disinfection efficiency of chlorine dioxide gas in student cafeterias in Taiwan

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In Taiwan, the food and drink requirements of students and faculty members are met by student cafeterias. The air quality within these cafeterias should satisfy the guidelines laid down by the Taiwan Environmental Protection Agency (Taiwan EPA). Accordingly, this study performed an experimental investigation into the efficiency of two different gaseous chlorine dioxide (ClO_2) treatments in disinfecting a local student cafeteria, namely a single, one-off application and a twice-daily application. In both cases, the ClO_2 was applied using strategically placed aerosol devices. The air quality before and after disinfection was evaluated by measuring the bioaerosol levels of bacteria and fungi. Moreover, a stepwise discriminant analysis method was applied for predicting the residual concentrations of bacteria and fungi, as a function of the environmental parameters and the ClO_2 concentration. The experimental results showed that the average background levels of bacteria and fungi prior to ClO_2 disinfection were 972.5 ± 623.6 and 1534.1 ± 631.8 colony-forming units (CFU)/m^3, respectively. A single ClO_2 application was found to reduce the bacterial and fungal concentration levels by as much as 65% and 30%, respectively. By contrast, a twice-daily ClO_2 application was found to reduce the bacterial and fungal concentration levels by as much as 74% and 38%, respectively. The statistical analysis results showed that the residual bacterial concentration level was determined primarily by the number of individuals present in the cafeteria, the temperature, and the ClO_2 concentration, whereas the residual fungal concentration level was determined mainly by the temperature, the total number of suspended particles, and the ClO_2 concentration. Thus, the integrated results suggest that the air quality guidelines prescribed by the Taiwan EPA for student cafeteria can best be achieved by applying ClO_2 twice daily using an appropriate deployment of aerosol devices.

Implications: ClO_2 gas can destroy all manner of microorganisms, including bacteria, spores, fungi, viruses, and even protozoans, in indoor environments. Moreover, it is popularly known that bioaerosols are able to grow and propagate on a wide variety of building materials and indoor surfaces. Thus, through optimal ClO_2 disinfection methodology, the indoor microbial contaminants can be decreased and the residual concentrations of bacteria and fungi as a function of the environmental parameters and the ClO_2 concentration can be predicted via some statistical techniques.

Introduction

Particles of biological origin account for approximately 24% of the total concentration of airborne particles (Matthias-Maser and Jaenicke, 1995; Jones and Harrison, 2004). Such particles result in a deterioration of the air quality and are thus of particular concern in confined, indoor environments, where their concentrations may exceed the levels regarded as safe for human health. Previous studies have shown a strong correlation between sick building syndrome (SBS) and indoor air pollution (Sanchez et al., 1987; Burge, 2004; Jaakkola et al., 2007). Specifically, the onset of SBS, which comprises a series of symptoms such as eye irritation, airway dryness, headaches, sleepiness, skin rashes, and so on, has been attributed to the presence of biological microbes or their components (World Health Organization [WHO], 2002; Mendell et al., 2008). As a result, the effects of microbial contamination of indoor air on human health and the development of suitable control measures have attracted growing attention in recent years (Orsini et al., 2002; Douwes et al., 2003; Adhikari et al., 2004; Jones and Harrison, 2004).

In general, the concentration and size distributions of indoor bioaerosols depend on a wide range of biotic and abiotic factors. For example, previous studies have shown that the moisture content of building materials, the relative humidity and temperature of the local environment, the air exchange rate, the presence of human activities, and the number of people and pets all significantly affect the concentration level of indoor bioaerosols (Kulmala et al., 1999; Buttner and Stetzenbach, 1993; American Conference of Governmental Industrial Hygienists [ACGIH], 1999). In nonindustrial indoor environments, airborne bacteria are generated mainly by the presence of humans and related activities such as talking, sneezing, coughing, walking, washing, toilet flushing, and so forth (Stetzenbach, 1997). Thus, although

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indoor environments are thought to be protective, they may in fact be contaminated with particles that present different and sometimes more serious risks than those encountered in outdoor environments, if their concentration levels exceed recommended safety limits.

According to the U.S. National Institute of Occupational Safety and Health (NIOSH) and the ACGIH, the total number of bioaerosol particles in indoor environments should not exceed 1000 colony-forming units [CFU]/m^3, whereas the total culturable count for bacteria should be no higher than 500 CFU/m^3 (ACGIH, 1989; American Industrial Hygiene Association [AIHA], 1996). In Taiwan, the indoor air quality should conform to the guidelines prescribed by the Environmental Protection Agency (Taiwan EPA) (Ling et al., 2008). For schools, educational facilities, playgrounds, hospitals, clinics, and health care facilities for the elderly and disabled, the indoor bacterial concentration should be no higher than 500 CFU/m^3, whereas that of fungi should not exceed 1000 CFU/m^3 (Taiwan EPA, 2011). According to the results of one long-term monitoring study, the level of biological contamination in Taiwan is much higher than the value of 1000 CFU/m^3 recommended by the WHO (Lin et al., 2007). Thus, to satisfy the Taiwan EPA guidelines for the air quality in indoor environments, effective disinfection treatments are required.

In Taiwan, the food and drink requirements of students and faculty members are met by student cafeterias. Such cafeterias are characterized by a high level of human activity and are conducive to the generation and propagation of a large number of bioaerosols by their very nature. As a result, stringent disinfection protocols are required to ensure the health of the cafeteria's occupants. The generation of gaseous chlorine dioxide (ClO_2) is one of several techniques available for the remediation of structures impacted by microbial growth (U.S. Environmental Protection Agency [EPA], 2007). ClO_2 can destroy all manner of microorganisms, including bacteria, spores, fungi, viruses, and even protozoans (Taylor and Butler, 1982; Chen and Vaughn, 1990; Sivaganesan et al., 2003; Loret et al., 2005). ClO_2 dissolves readily in water, forming a stable state of small particles. Under room temperature conditions, the ClO_2 content within the water evaporates and propagates naturally through the local environment, providing a disinfection function. Being in a gaseous form, the ClO_2 molecules are able to penetrate deeply into building cavities, wall cavities, and other hard-to-access areas, and therefore provide an extremely thorough sterilization function (Buttner et al., 2004; Han et al., 2003). Gaseous ClO_2 has been approved by the EPA as a disinfectant, sanitizer, and sterilant for the paper, fruit, vegetable, dairy, poultry and beef processing industries (EPA, 2000), and for the processing of industrial wastewater (Lee et al., 2006). Furthermore, aqueous ClO_2 is commonly used for the treatment of drinking water and for the control of mold in libraries (Southwell, 2002; Weaver-Meyers et al., 1998). Following the 9–11 attacks in the USA, ClO_2 gas was used to treat the *Bacillus anthracis* spores detected in certain government buildings and on the exterior of mail packages addressed to a small number of government institutions (EPA, 2006; Canter et al., 2005). Finally, various studies have demonstrated the efficiency of ClO_2 in deactivating *Bacillus* endospores (surrogates for *B. anthracis* spores) (Buttner et al., 2001; Cortezzo et al., 2004; Young and Setlow, 2003).

In our previous study (Hsu et al., 2013), we found that a one-off ClO_2 application yielded a 12-hr disinfection efficiency of less than 26.0% in a local cafeteria in Taiwan. In performing the disinfection process, the ClO_2 solution (0.3 mg/m^3) was simply placed in unsealed containers and allowed to evaporate and propagate naturally through the environment. In this condition, air samples were collected from five sampling points in the indoor area of the cafeteria. According to the results of the one-way analysis of variance (ANOVA) test, the sampling data in the indoor area of the cafeteria showed no significant difference ($p > 0.05$). Therefore, in the present study sampling was conducted in single location within the cafeteria and a more rigorous disinfection treatment was performed, in which ClO_2 was applied via strategically placed aerosol devices. Two different ClO_2 disinfection protocols were considered: namely, a single, one-off application and a twice-daily application. The air quality in the cafeteria before and after ClO_2 disinfection was evaluated in terms of the bioaerosol levels of bacteria and fungi.

Moreover, we know that discriminant statistical analysis can be used, firstly, to predict group membership for new cases, especially when there are more than two groups and, secondly, to provide a visual representation of structure underlying the relationships among large numbers of variables and groups (Savić et al., 2008). Therefore, for clarifying the influencing parameters of the air quality in student cafeterias, we used a stepwise discriminant analysis method to determine the relative disinfection efficiencies of the two different methods and to develop analytical formulae for predicting the residual concentrations of bacteria and fungi as a function of the environmental parameters and the ClO_2 concentration. Finally, according to the total results, we will recommend a good ClO_2 disinfection method in such an environment.

Materials and Methods

The study was conducted in the student cafeteria at Chia-Nan University in southern Taiwan. Prior to disinfection, air samples were collected and analyzed in order to determine the background concentration levels of bacteria and fungi. ClO_2 disinfection was then carried out using the two different application procedures described above. On each sampling day, air samples were collected over a 12-hr period in order to evaluate the reduction in the bacterial and fungal concentration levels. The details of the experimental procedure are described in the sections below.

Study area and sampling time

Figure 1 presents the floor plan of the student cafeteria where only a single sampling point is selected and marked. The reason is that all five sampling points installed at different locations inside the cafeteria provided no significant difference in air sample characteristics, as shown in our previous study (Hsu et al., 2013). The experimental investigation was conducted over a 12-month period (i.e., June 2010∼May 2011). On each sampling day, air samples were collected on an hourly basis

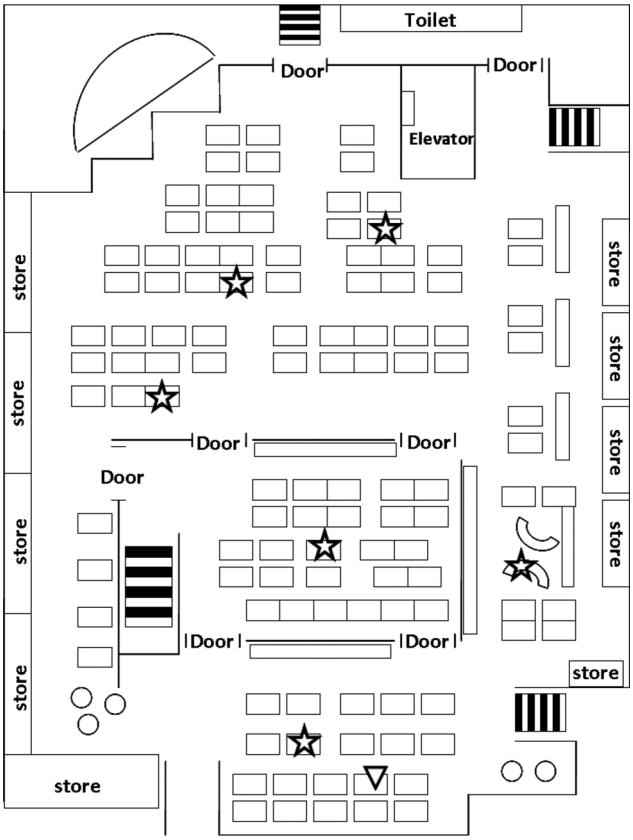

Figure 1. Floor plan of student cafeteria at Chia-Nan University of Pharmacy and Science, Taiwan. ☆ = location of ultrasonic aerosol devices; ▽ = sample collection location.

between 8:00 a.m. and 8:00 p.m. The samples were then analyzed in order to determine the concentrations of the various biological (i.e., bacterial and fungal) and nonbiological (i.e., CO_2) components. To ensure the reliability of the analysis results, each sample was tested in triplicate. To investigate the effects of the environmental factors on the disinfection efficiencies of the two methods, the relative humidity and temperature were measured each time a sample was collected using a TES-1364 humidity temperature meter (TES Corp., Taipei, Taiwan). The Q-TRAK Indoor Air Quality (IAQ) meter (model 7565; TSI Inc., St. Paul, MN, USA) used to measure the air flow velocity and CO_2 concentration. Moreover, the total number of suspended particles (TSP) was measured using an Aerocet-531 mass particle counter (Met One Instruments, Inc., Grants Pass, OR, USA). Finally, a record of the number of individuals present within the cafeteria at the time the sample was collected.

Disinfection methods

According to the U.S. Occupational Safety and Health Administration (OSHA) and the ACGIH, the 8-hr time-weighted average (TWA) of ClO_2 in the workplace should not exceed 0.3 mg/m^3 (equivalent to 0.1 mg/L) (OSHA, 2006). Meanwhile, the 15-min short-term exposure limit (STEL) of ClO_2 should not exceed 0.9 mg/m^3 (equivalent to 0.3 mg/L) (OSHA, 2006). According to the design blueprints of the cafeteria, the cafeteria had a volume of 2375 m^3. Thus, to satisfy the 8-hr TWA limit of 0.3 mg/m^3, disinfection was performed using 250 mg/L ClO_2 solution. In performing the disinfection process, the ClO_2 solution was equally divided among six ultrasonic aerosol devices (i.e., less than 0.475 L per container), which were then placed at the six locations shown in Figure 1. At temperatures of 11 °C or higher, the free radical of ClO_2 occurs in gaseous form (Han et al., 2003). Because the average temperature within the cafeteria was always higher than 11 °C during the experimental period, the ClO_2 solution evaporated immediately following nebulization and then propagated naturally through the local environment.

On each sampling day, disinfection was performed at 10:00 a.m. and air samples were then collected on an hourly basis until 8:00 p.m. As described above, two different disinfection modes were considered, namely a single-application mode (SAM) and a twice-daily application mode (TAM). In the SAM disinfection, the ClO_2 solution was applied for 1 day only, and was not replenished as it nebulized. In the TAM disinfection, the ClO_2 solution was also applied for 1 day only, but was replenished after 5 hr.

Air sample collection

Air samples with a volume of 1000 L were collected at a single sampling point (see Figure 1) in accordance with the Taiwan National Institute of Environmental Analysis (NIEA) guidelines (i.e., NIEA E301.11C for bacteria and E401.11C for fungus; Taiwan EPA, 2008). The samples were collected using a MAS-100 Eco Microbial Air Sampler (Merck, Darmstadt, Germany; 100 L/min) containing Petri dishes with tryptic soy agar (TSA) plates and malt extract agar (MEA) plates. Following a 10-min collection period, the Petri dishes were removed from the sampler in order to cultivate the bioaerosols. For the bacterial bioaerosols, the TSA plates were incubated at a temperature of 30 ± 1 °C for 48 ± 2 hr. Meanwhile, for the fungal bioaerosols, the MEA plates were incubated at 25 ± 1 °C for 4 ± 1 days. The bacterial and fungal concentrations were then evaluated by counting the colonies formed on the respective agar surfaces.

Statistical analysis

The sample data were analyzed using the SPSS statistical package (version 12, 2003; SPSS Inc., Chicago, IL, USA). All of the data collected in the study were shown by the Shapiro-Wilks test to satisfy the assumption of normality. The correlation between the colony count and the environmental parameters (i.e., temperature, relative humidity, airflow velocity, CO_2 level, TSP, and number of individuals in the cafeteria) was examined by means of a Pearson's correlation analysis. In addition, a one-way ANOVA test was used to test for significant differences in the relative effects of the environmental parameters on the formation of the bacterial and fungal colonies in the semester and vacation periods. Moreover, a Duncan's multiple-range test was used to test for significant differences in the respective effects of the two disinfection methods on the formation of the bacterial and fungal colonies in the semester and vacation periods (ANOVA, $\alpha = 0.05$; SPSS). To estimate the effects of the two disinfection

protocols on the residual concentrations of bacteria and fungi, the following regression formulae were constructed to relate the residual bacterial and fungal concentrations with the environmental and experimental variables:

$$A_i = \alpha_0 + \beta_1 \times (\text{no. of individuals})_i + \beta_2 \times (\text{temperature})_i + \beta_3 \times (\text{relative humidity})_i + \beta_4 \times (\text{airflow velocity})_i + \beta_5 \times (\text{CO}_2 \text{ concentration})_i + \beta_6 \times (\text{TSP})_i + \beta_7 \times (\text{ClO}_2 \text{ concentration})_i$$

$$B_i = \alpha_0 + \beta_1 \times (\text{no. of individuals})_i + \beta_2 \times (\text{temperature})_i + \beta_3 \times (\text{relative humidity})_i + \beta_4 \times (\text{airflow velocity})_i + \beta_5 \times (\text{CO}_2 \text{ concentration})_i + \beta_6 \times (\text{TSP})_i + \beta_7 \times (\text{ClO}_2 \text{ concentration})_i$$

where A_i denotes the residual bacterial concentration (CFU/m^3); B_i is the residual fungal concentration (CFU/m^3); α_0 is a constant; and β_i is the regression coefficient of the ith variable. A stepwise discriminant analysis procedure (one variable removed, $P > 0.1$) was then applied to filter out the least significant variables such that two simplified expressions for the residual bacterial and fungal concentrations were obtained.

Results and Discussion

Air quality in cafeteria

As discussed earlier, indoor environments can potentially pose a greater risk to human health than the outdoor environment because the enclosed space can lead to a dangerously high accumulation of bioparticles and other harmful contaminants. Previous research has shown that the indoor air quality is affected by a number of factors, including the ambient temperature, the relative humidity, the air exchange rate, the air velocity, the degree of ventilation, the level of human activity, and so on (Tsai et al., 2010; Yu et al., 2009). As discussed above, the experimental investigation performed in this study was conducted over a period of 12 months (June 2010∼May 2011). In other words, the indoor air quality was investigated during both semester and vacation periods. Because the semester and vacation periods are characterized by a large difference in the degree of human activity, the sampling results given in the remainder of this paper are presented separately for the semester and vacation periods.

Table 1 summarizes the measurement results (mean ± SD) obtained in the semester and vacation periods for the relative humidity, temperature, airflow velocity, CO_2 level, TSP level, number of individuals in the cafeteria, ClO_2 concentration, residual bacterial concentration, and residual fungal concentration given the two disinfection protocols, namely SAM (single-application mode) and TAM (twice-daily application mode). In the vacation period, the average temperature during the SAM and TAM application procedures was 22.3 ± 0.3 and 16.4 ± 0.4 °C, respectively. The corresponding relative humidity values were 73.3% ± 0.9% and 67.7% ± 1.6%, whereas the corresponding airflow velocities were 2.68 ± 0.24 and 0.46 ± 0.40 ft/min. Similarly, in the semester period, the average temperature during the SAM and TAM application procedures was 22.9 ± 0.4 and

Table 1. Experimental conditions before and after ClO_2 treatment in the vacation and semester periods (mean ± SD)

		After ClO_2 Treatment			
		Vacation		Semester	
Items	Before ClO_2 Treatment	SAM	TAM	SAM	TAM
Number of samples	52	221	78	78	39
Number of individuals present	2.0 ± 1.7^a	3.4 ± 0.8^a	2.7 ± 1.4^a	69.1 ± 1.4^b	87.2 ± 1.9^c
Temperature (°C)	26.8 ± 0.5^a	22.3 ± 0.3^b	16.4 ± 0.4^c	22.9 ± 0.4^b	23.2 ± 0.6^b
Relative humidity (%)	87.7 ± 1.9^a	73.3 ± 0.9^b	67.7 ± 1.6^c	57.5 ± 1.6^d	58.4 ± 2.3^d
Airflow velocity (ft/min)	0.79 ± 0.49^a	2.68 ± 0.24^b	0.46 ± 0.40^a	6.10 ± 0.40^c	3.26 ± 0.58^b
Carbon dioxide (ppm)	297.2 ± 10.4^a	255.6 ± 05.0^b	239.9 ± 08.5^b	333.3 ± 08.5^c	389.8 ± 11.9^c
TSP (mg/m^3)	0.13 ± 0.10^a	0.31 ± 0.05^{ab}	0.32 ± 0.08^{ab}	0.42 ± 0.08^b	0.34 ± 0.12^{ab}
Chlorine dioxide (mg/L)	0.00 ± 0.000	0.270 ± 0.175	0.257 ± 0.000	0.257 ± 0.000	0.257 ± 0.000
Residual bacteria (CFU/m^3)	972.5 ± 623.6^a	339.4 ± 257.1^c	249.6 ± 145.9^c	534.6 ± 372.3^b	469.1 ± 190.1^b
Total disinfected bacteria (%)	—	65.1	74.3	45.0	51.8
Residual fungi (CFU/m^3)	1534.1 ± 631.8^a	1063.8 ± 604.8^b	952.8 ± 303.4^b	1026.0 ± 421.9^b	1077.8 ± 469.6^b
Total disinfected fungi (%)	—	30.7	37.9	33.1	29.7

Note: Within the same column, entries annotated with different superscript letters exhibit a statistical difference according to Duncan ANOVA test ($p < 0.05$).

23.2 ± 0.6 °C, respectively. The corresponding relative humidity values were 57.5% ± 1.6% and 58.4% ± 2.3%, whereas the corresponding airflow velocities were 6.10 ± 0.40 and 3.26 ± 0.58 ft/min. Finally, the number of individuals present during the SAM and TAM application procedures in the vacation period was 3.4 ± 0.8 and 2.7 ± 1.4, respectively, whereas in the semester period the corresponding number of individuals was 69.1 ± 1.4 and 87.2 ± 1.9. The results presented in Table 1 show that the number of individuals present in the cafeteria at the time the samples were collected is significantly lower in the vacation period than in the semester period because the cafeteria is closed for business during the vacation. As shown in Table 2, the results show that a positive correlation exists between the residual bacterial concentration and the indoor temperature (bacteria: $r = 0.366$, $p < 0.01$) and the number of individuals present (bacteria: $r = 0.153$, $p < 0.01$) and relative humidity (bacteria: $r = 0.192$, $p < 0.01$; fungi: $r = 0.184$, $P < 0.01$) and carbon dioxide (bacteria: $r = 0.207$, $p < 0.01$). A negative correlation exists between the residual fungal concentration and the indoor temperature (fungi: $r = -0.228$, $p < 0.01$), between the residual fungal concentration and airflow velocity (fungi: $r = -0.274$, $p < 0.01$), and between the residual fungal concentration and TSP (fungi: $r = -0.168$, $p < 0.01$). The results show that the residual bacterial concentration increases with an increasing temperature or an increasing number of individuals or an increasing relative humidity or an increasing carbon dioxide within the cafeteria. The residual fungal concentration decreases with an increasing temperature or an increasing airflow velocity or an increasing TSP within the cafeteria. However, a negative correlation exists between the residual bacterial and fungal concentrations and the ClO_2 concentration (bacteria: $r = -0.334$, $p < 0.01$; fungi: $r = -0.482$, $p < 0.01$). The results confirm the efficacy of the two disinfection protocols in reducing the residual levels of bacteria and fungi in the cafeteria. That is because airflow velocity is conducive to the spread of ClO_2, so it can propagate naturally through the environment. Thus, airflow velocity has significant impact on ClO_2 concentration.

Table 1 shows the average bacterial and fungal concentration levels in the cafeteria before and after ClO_2 treatment. Note that the annotation "before" refers to the samples collected in a preliminary investigation performed prior to the disinfection experiments, whereas the annotation "after" refers to the samples collected during the experimental stage of the investigation in which ClO_2 was applied using the SAM and TAM disinfection methods. As shown, the average concentrations of bacteria and fungi prior to disinfection were 972.5 ± 623.6 and 1534.1 ± 631.8 CFU/m^3, respectively. These concentration levels are higher than the recommended levels prescribed by the Taiwan EPA (i.e., 500 and 1000 CFU/m^3, respectively; Taiwan EPA, 2011). The results confirm the need for ClO_2 disinfection in order to ensure that the air quality within the cafeteria complies with Taiwan EPA standards.

As shown in Table 1, the residual bacterial concentration following the SAM and TAM disinfection methods was respectively 339.4 ± 257.1 and 249.6 ± 145.9 CFU/m^3 in the vacation period, and 534.6 ± 372.3 and 469.1 ± 190.1 CFU/m^3 in the semester period. Similarly, the residual fungal concentration following the SAM and TAM disinfection methods was respectively 1063.8 ± 604.8 and 952.8 ± 303.4 CFU/m^3 in the vacation period, and 1026.0 ± 421.9 and 1077.8 ± 469.6 CFU/m^3 in the semester period. According to the results obtained from the Duncan ANOVA test, the residual bacterial and fungal concentrations in the semester period are significantly higher than those in the vacation period ($p < 0.05$). Moreover, in both periods, the residual bacterial and fungal concentrations following SAM disinfection are significantly higher than those following TAM disinfection ($p < 0.05$). In general, the results show that the application of ClO_2 yields an effective reduction in both the residual bacterial concentration and the residual fungal concentration. The reduction is particularly apparent in the case of the bacterial concentration. Moreover, it is seen that of the two methods, the TAM application method generally yields a greater disinfection efficacy. However, a negative correlation exists between the residual fungal concentration and airflow velocity within the cafeteria. The airflow velocity following the SAM and TAM

Table 2. The correlation between the colony count and the environmental parameters

Items	No. of Individuals Present	Temperature	Relative Humidity	Airflow Velocity	Carbon Dioxide	TSP	Residual Bacteria	Residual Fungi
Temperature	0.113*							
Relative humidity	−0.257**	0.129**						
Airflow velocity	0.249**	0.349**	−0.473**					
Carbon dioxide	0.486**	0.319**	−0.080	0.102*				
TSP	−0.038	0.007	−0.183**	0.020	−0.031			
Residual bacteria	0.153**	0.366**	0.192**	−0.006	0.207**	0.029		
Residual fungi	−0.037	−0.228**	0.184**	−0.274**	−0.075	−0.168**	0.169**	
Chlorine dioxide	0.098*	−0.264**	−0.418**	0.240**	−0.052	0.095*	−0.482**	−0.334**

Notes: **Correlation is significant at the 0.01 level (two-tailed). *Correlation is significant at the 0.05 level (two-tailed).

was respectively 6.10 ± 0.40 and 3.26 ± 0.58 ft/min in the semester period. The results show that the airflow velocity difference results in the total disinfected fungal percent of the SAM is higher than that of the TAM. However, the residual fungal concentration decreases with an increasing airflow velocity and TSP within the cafeteria. Therefore, the SAM method outperforms the TAM method in disinfecting fungi during the semester period.

In Table 1, it can be seen that the SAM and TAM application methods reduce the original bacterial concentration by respectively 65.1% and 74.3% in the vacation period, and by 45.0% and 51.8% in the semester period. Similarly, the SAM and TAM application methods reduce the residual fungal concentration by respectively 30.7% and 37.9% in the vacation period, and by 33.1% and 29.7% in the semester period. The reduction in the residual contaminant concentration level is significant in every case. The results confirm that the TAM disinfection method yields a greater average disinfection efficiency (48.4%) than the SAM disinfection method (43.6%). Furthermore, the TAM application method reduces the concentration levels of both contaminants to a level consistent with (or very close to) the Taiwan EPA guidelines. Finally, the results show that, in general, the disinfection efficiencies of the two methods are improved during the vacation period due to the corresponding reduction in human activity.

Stepwise discriminant analysis

The correlation between the colony count and the environmental parameters was already shown in Table 2. The stepwise discriminant analysis procedure (one variable removed, significance level $p > 0.1$) was applied to identify the environmental and experimental parameters having the greatest effect on the residual bacterial and fungal concentration levels. The results showed that the residual bacterial concentration level was determined primarily by the number of individuals present in the cafeteria, the temperature, and the ClO_2 concentration, whereas the residual fungal concentration level was determined mainly by the temperature, the total number of suspended particles, and the ClO_2 concentration. Moreover, the coefficients in the two regression formulae for the residual bacterial and fungal concentrations were shown to be as follows:

$$A_i = 470.0702 + 1.249561 \times (\text{no. of individuals})_i + 18.74183 \times (\text{temperature})_i - 1989.13 \times (\text{C1O}_2\ \text{concentration})_i \quad (1)$$

$$B_i = 2651.472 - 40.755 \times (\text{temperature})_i - 87.3871 \times (\text{TSP})_i - 2681.76 \times (\text{C1O}_2\ \text{concentration})_i \quad (2)$$

Utilizing a holdout sample cross-validation technique, the residual bacterial concentration was found to be 751 CFU/m^3 with no people present in the cafeteria, 0 mg/L of ClO_2, and a temperature of 15 °C (see Table 3). Similarly, the residual bacterial concentration was found to be 814 CFU/m^3 given 50 people present, 0 mg/L of ClO_2, and a temperature of 15 °C; 939 CFU/m^3 given no people present, 0 mg/L of ClO_2, and a temperature of 25 °C; and 1001 CFU/m^3 given 50 people present, 0 mg/L of ClO_2, and a temperature of 25 °C. Given a ClO_2 concentration of 0.3 mg/L, the residual bacterial concentration was found to be 154 CFU/m^3 given no people present and a temperature of 15 °C; 217 CFU/m^3 given 50 people present and a temperature of 15 °C; 342 CFU/m^3 given no people present and a temperature of 25 °C; and 404 CFU/m^3 given 50 people present and a temperature of 25 °C. In other words, the residual bacterial concentration decreases with an increasing ClO_2 concentration, but increases with both an increasing number of individuals in the cafeteria and an increasing temperature.

Utilizing the same holdout sample cross-validation technique, the residual fungal concentration was found to be 2040 CFU/m^3 given a ClO_2 concentration of 0 mg/L, a TSP concentration of 0 mg/m^3, and a temperature of 15 °C; 1996 CFU/m^3 given a ClO_2 concentration of 0 mg/L, a TSP concentration of 0.5 mg/m^3, and a temperature of 15 °C; 1633 CFU/m^3 given a ClO_2 concentration of 0 mg/L, a TSP concentration of 0 mg/m^3, and a temperature of 25 °C; and 1589 CFU/m^3 given a ClO_2 concentration of 0 mg/L, a TSP concentration of 0.5 mg/m^3, and a temperature of 25 °C (see Table 4). Given a ClO_2 concentration of 0.3 mg/L, the residual fungal concentration was found to be 1236 CFU/m^3 given a TSP concentration of 0 mg/m^3 and a temperature of 15 °C; 1192 CFU/m^3 given a TSP concentration of 0.5 mg/m^3 and a temperature of 15 °C; 828 CFU/m^3 given a TSP of 0 mg/m^3 and a temperature of 25 °C; and 784 CFU/m^3 given a TSP of 0.5 mg/m^3 and a temperature of 25 °C. In other words, the residual fungal concentration decreases with an increasing ClO_2 concentration, an increasing TSP concentration, and an increasing temperature.

Table 3. Factor analysis: Variables affecting bacterial concentration in cafeteria

		Bacterial Concentration (CFU/m^3)		
Items	No. of Individuals Present	15 °C	22.5 °C	25 °C
0 mg/L ClO_2	0	751	892	939
	50	814	954	1001
0.2 mg/L ClO_2	0	353	494	541
	50	416	556	603
0.3 mg/L ClO_2	0	154	295	342
	50	217	358	404

Table 4. Factor analysis: Variables affecting fungal concentration in cafeteria

Items	TSP (mg/m^3)	Fungal Concentration (CFU/m^3)		
		15 °C	22.5 °C	25 °C
0 mg/L ClO_2	0	2040	1734	1633
	0.5	1996	1691	1589
0.2 mg/L ClO_2	0	1504	1198	1096
	0.5	1460	1154	1053
0.3 mg/L ClO_2	0	1236	930	828
	0.5	1192	886	784

From eq 1, the residual bacterial concentration is estimated to be 469 CFU/m^3 given the use of the TAM disinfection method with 87 people present in the cafeteria, a temperature of 23.2 °C, and a ClO_2 concentration of 0.257 mg/L. This estimated result is in good agreement with the experimental measurement (i.e., 469.1 ± 190.1 CFU/m^3; see Table 1). From eq 2, the residual fungal concentration is estimated to be 1078 CFU/m^3 given the use of the TAM disinfection method with 87 people present, a temperature of 23.2 °C, and a ClO_2 concentration of 0.257 mg/L. This estimated result is also in good agreement with the experimental measurement (i.e., 1077.8 ± 469 CFU/m^3; see Table 1). In other words, the basic validity of the regression formulae presented in eqs 1 and 2 for the residual bacterial concentration and residual fungal concentration is confirmed.

Bacterial and fungal concentration levels before and after ClO_2 treatment

Figures 2 and 3 show the hourly variation in the bacterial and fungal concentration levels in the student cafeteria during the semester and vacation periods given the use of the SAM and TAM disinfection protocols. Note that as in Table 1, the annotation "before" refers to the samples collected in a preliminary investigation prior to the disinfection experiments, whereas the annotation "after" refers to the samples collected during the disinfection experiments. As shown in Figures 2 and 3, the average bacterial and fungal concentrations in the cafeteria prior to disinfection were 972.5 ± 623.6 and 1534.1 ± 631.8 CFU/m^3, respectively. These concentration levels are higher than the maximum permissible levels prescribed by the Taiwan EPA (i.e., 500 and 1000 CFU/m^3, respectively; Ling et al., 2008; Taiwan EPA, 2011). The results confirm the need for ClO_2 disinfection in order to ensure that the air quality within the cafeteria complies with Taiwan EPA standards.

It is seen that the bacterial and fungal contaminants both have a relatively low concentration at 8:00 a.m. due to settlement overnight. However, during the course of the day, the background bacterial concentration level increases and varies significantly as a result of activities such as cooking, cleaning, eating, and the movement of people. This finding confirms that the concentration of airborne bacteria in indoor settings is determined primarily by the presence of humans (Council of the European Community [CEC], 1993). As commented above,

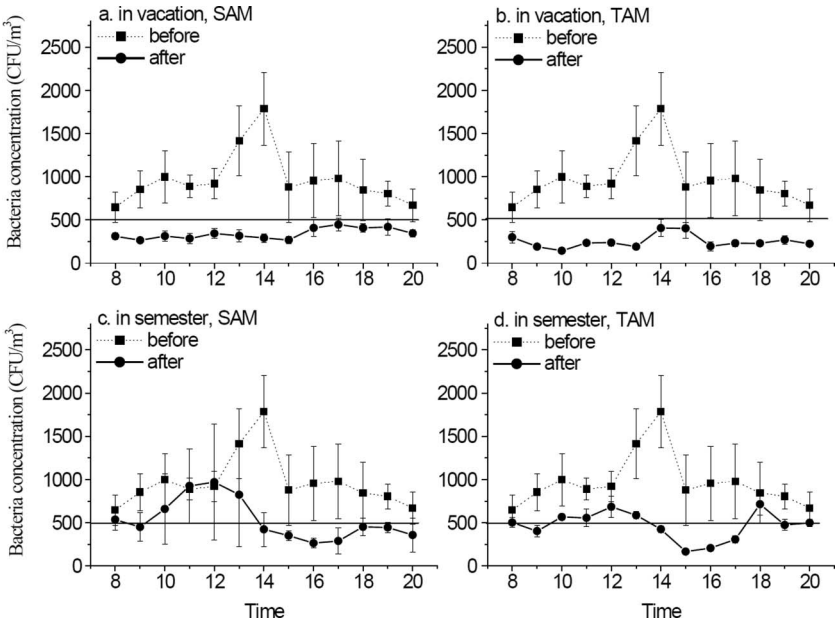

Figure 2. Impact of the two disinfection methods on indoor bacterial bioaerosol concentration during semester and vacation periods.

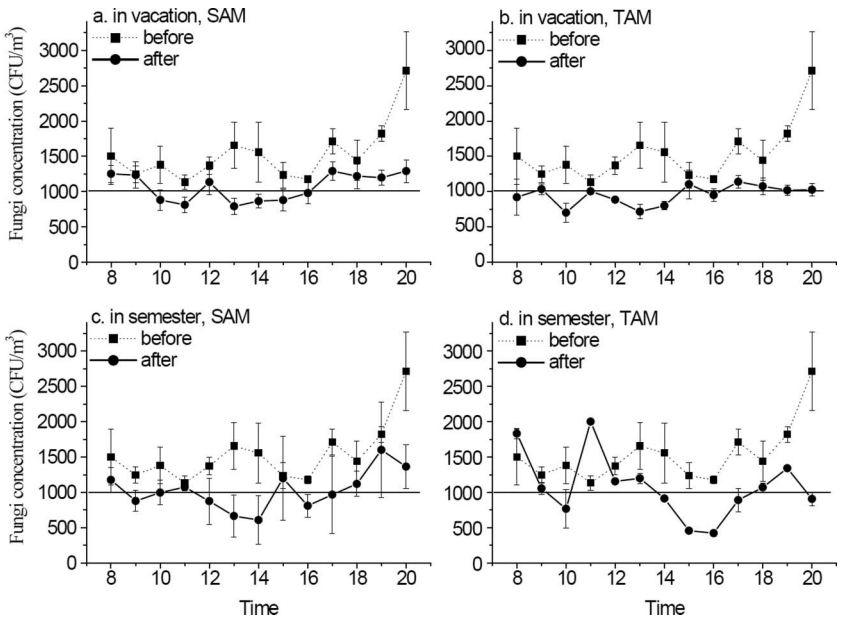

Figure 3. Impact of the two disinfection methods on indoor fungal bioaerosol concentration during semester and vacation periods.

the bacterial concentration is higher than the value of 500 CFU/m^3 recommended by the Taiwan EPA. During the vacation period, both ClO_2 treatments reduce the concentration level to a value less than the recommended one (see Figure 2a and b). Comparing the two panels, it is seen that the bacterial concentration following TAM disinfection is less than that following SAM disinfection. Figure 2c and d shows that during the semester, the bacterial concentration is significantly higher than the recommended value of 500 CFU/m^3 at virtually all of the sampling times (particularly during the peak dining hours of 10.00∼2.00 p.m. and 6.00 p.m.). By contrast, for the residual bacterial concentration per hour, SAM the standard deviation (SD) of the bacterial concentration is all greater than TAM the SD of the bacteria concentration by examining each point of the "after" line in SAM and TAM conditions. Thus, the single ClO_2 treatment (i.e. SAM) has little effect in improving the air quality within the cafeteria. In other words, it is inferred that the TAM method provides an improved disinfection performance.

As shown in Figure 3, both disinfection methods reduce the fungal concentration levels in the vacation and semester periods. However, the residual fungal concentration level is still slightly higher than the maximum permissible limit prescribed by the Taiwan EPA (1000 CFU/m^3) in most cases. In other words, the two disinfection protocols are less effective in reducing the fungal concentration level than in reducing the bacterial concentration level. In addition, it is seen that there is little difference in the efficiencies of the two disinfection protocols in the semester period and the vacation period, respectively. The results support the findings of the stepwise discriminant analysis, which shows that the residual fungal concentration is not greatly influenced by the number of individuals in the cafeteria (see eq 2). As shown in Figure 3, for the residual fungal concentration the hourly, SAM the standard deviation (SD) of the fungal concentration level is mostly greater than TAM the SD of the fungal concentration by examining each point of the "after" line in SAM and TAM conditions. Therefore, the SAM treatment protocol has little effect in improving the air quality. It is inferred that of the two disinfection methods, the TAM method has a greater efficiency in reducing the residual fungal concentration.

Overall, the results presented in this study show that for both the semester period and the vacation period, the residual bacterial and fungal concentrations following disinfection are significantly lower than those before disinfection. In addition, the disinfection efficiency of the TAM method is generally better than that of the SAM method. For both methods, the ClO_2 disinfection process results in a more effective reduction in the bacterial concentration level than in the fungal concentration level. For both disinfection methods, the residual bacterial concentration level in the semester period is significantly higher than that in the vacation period. The results confirm that cooking, cleaning, and the passage of individuals are major sources of indoor bioaerosols (Abt et al., 2000). In the vacation period, both disinfection methods reduce the residual bacterial concentration to a level complying with the Taiwan EPA guidelines for the air quality in student cafeterias. However, during the semester period, a single application of ClO_2 is insufficient to satisfy the Taiwan EPA requirement and should be replaced by a twice-daily application.

Conclusion

This study has performed an experimental investigation into the efficacy of ClO_2 as a disinfection agent for student cafeterias in Taiwan. Two different ClO_2 application methods have been considered, namely a single, daily treatment and a twice-daily treatment. In both methods, disinfection was performed using a ClO_2 concentration of not exceeding 0.3 mg/m^3. Furthermore, the ClO_2 solution was nebulized using ultrasonic aerosol devices and then allowed to propagate naturally through the local environment. The experimental results support the following major conclusions:

(a) From our statistical analysis results, we can infer that the residual bacterial concentration following disinfection is determined primarily by the number of individuals present in the cafeteria, the temperature, and the ClO_2 concentration, whereas the residual fungal concentration level is determined mainly by the temperature, the total number of suspended particles, and the ClO_2 concentration.
(b) Both disinfection methods yield a reduction in the bacterial and fungal concentration levels during the semester and vacation periods. However, in general, the two methods are more effective in reducing the bacterial concentration level than the fungal concentration level.
(c) In general, both disinfection methods reduce the residual bacterial and fungal concentration levels to a level consistent with (or very close to) the Taiwan EPA guidelines. However, in the semester period, the single-application method fails to meet the Taiwan EPA guidelines for the bacterial concentration level. Thus, overall, the present results suggest that the twice-daily application method provides a more robust protocol for meeting the Taiwan EPA guidelines for the indoor air quality in student cafeterias in Taiwan.
(d) The present study has focused on the specific case of student cafeterias in Taiwan. However, it is reasonable to infer that the findings presented in this study regarding the efficacy of a twice-daily ClO_2 application can be extended to the case of all education institutions, health care facilities, and public areas, where the reduction of bioaerosols are of particular concern.

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