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In Situ Rapid Evaluation of Indoor Bioaerosols Using an ATP Bioluminescence Assay

Chien-Jung Lin[1], Yi-Tun Wang[1], Kuo-Jung Hsien[2], Ying I. Tsai[1*], Pei-Yi Kung[1], Jih-Ming Chyan[1]

[1] *Department of Environmental Resources Management, Chia Nan University of Pharmacy and Science, 60, Sec. 1, Erren Rd., Rende, Tainan 71710, Taiwan*
[2] *Department of Environmental Engineering and Science, Chia Nan University of Pharmacy and Science, 60, Sec. 1, Erren Rd., Rende, Tainan 71710, Taiwan*

ABSTRACT

An adenosine-5'-triphosphate (ATP) bioluminescence method was developed for detecting microbial activity in indoor air. This method was compared with the traditional method of collecting, culturing and counting CFUs. A comparison of the results showed that ATP bioluminescence, expressed as RLUs, was moderately correlated with the entire set of CFU counts ($r = 0.607$), and that the correlation improved to $r = 0.963$ (p value < 0.001) when CFU outliers were removed from the calculations. The ATP bioluminescence method was applied at four different sites; a hospital Chinese medicine diagnostic room, a library, a government office, and a railway station lobby. The results showed that microbial activity was far higher in the railway station lobby than at the other three sites, and this is believed to be due to the higher volume and density of people in this space. At all four sites, higher microbial activity was linked to indoor plants, garbage cans, shoe racks, and furnished waiting areas. PCA of the data showed that microbial activity in the Chinese medicine diagnostic room was closely related to room temperature and humidity, and hence lowering the latter can reduce the potential for microbial activity at this site. At all four sites, no correlation was found between microbial activity and airborne pollutants. The ATP bioluminescence method was applied for the rapid evaluation of room disinfection using chloride dioxide, and results showed that twenty minutes after spraying with 100 ppm ClO_2, microbial activity was reduced to 38.7% of its original level. ATP bioluminescence is simpler, easier to operate, and more cost-effective than the conventional microbial culture method of evaluating microbial load. The results obtained in this research confirm that the proposed ATP bioluminescence technique is capable of instantaneously detecting microbial activity in an indoor environment. Moreover, this approach can be used for on-line evaluation of room disinfection efficiency.

Keywords: ATP bioluminescence; Bioaerosol; Microbial activity; Relative light unit; Principal component analysis; Indoor air quality.

INTRODUCTION

Adenosine-5'-triphosphate (ATP) exists in all living cells as a source of energy that can be easily stored and used when needed for cellular functions. The luminescence of fire flies is a well-known example of ATP energy transformation. ATP released by microbial cells produces the same light in the presence of luminescence enzymes and its intensity can be measured with a luminescence instrument and quantified as Relative Light Units (RLUs). The measured ATP reflects microbial activity and indirectly indicates the quantity of microorganisms present.

Microbial biomass can be quantified by measuring ATP via bioluminescence, and because of this ATP bioluminescence technology has been widely applied in many fields in which microbial load/activity is important, including restaurant hygiene (Griffiths, 1996), medical environments (Griffith *et al.*, 2000; Aycicek *et al.*, 2006; Cooper *et al.*, 2007), food and drink sanitation (Niza-Ribeiro *et al.*, 2000; Lehto *et al.*, 2011) drinking water quality (Delahaye *et al.*, 2003; Costa *et al.*, 2004), composting (Horiuchi *et al.*, 2003; Tiquia *et al.*, 2002), waste-activated sludge (Chu *et al.*, 2008), soil research (Contin *et al.*, 2000, 2001) and ambient air monitoring (Lee and Chang, 2000; Seshdri *et al.*, 2009).

When investigating ambient air inside buildings, it is important that the microbial load in the space is accurately represented. There is uncertainty as to the role of indoor air handling systems and building materials on the growth and

* Corresponding author. Tel.: 886-6-266-0208;
Fax: 886-6-266-0606
E-mail address: mtsaiyi@mail.chna.edu.tw

dissemination of microbial populations indoors and even the efficacy of biocides in mitigation of indoor air quality problems. Yang and Heinsohn (2007) recommended observing the space for places showing water marks due to leaking problems and traces of microbial growth, noting complaints made by residents or users of the space of discomfort, and preferentially selecting locations that are crowded with people. However, selection of locations for monitoring based on observation with the naked eye and anecdote is not objective and hence not convincing. Pre-sampling the space and evaluating the possible bioaerosol concentration range is preferable, in conjunction with reviewing of literature (Lee, 2011; Huang *et al.*, 2012). Meanwhile, concentrations of bacterial bioaerosols varied in public restroom environments during a long term of observation (Lee *et al.*, 2012). Furthermore, several bioaerosol monitoring methods for microorganisms in indoor air have been involved. These methods include impaction, filtration, impingement and depositional sampling. A rapid assessment method was developed to determine bacterial viability by applying ultraviolet and visible spectroscopy analysis (Park *et al.*, 2012). However, an evaluation of sensitivity, repeatability and accuracy for bioaerosol sampling is important and it is essential to determine the capabilities and limitations of the various available sampling methods. The results could be largely impacted by the differences from the use of different samplers (Buttner and Stetzenbach, 1991; Chen, 1997). Buttner and Stetzenbach (1993) demonstrated that even in the controlled environmental conditions of the experimental room, repeatability could be low for aerobiological samplers. The lack of data concerning the transport and fate of microorganisms in indoor air environments and the absence of standardized monitoring protocols have resulted in confusion in interpretation of survey data from office and residential environments.

The conventional culture technology for studying bioaerosols is time-consuming and costly as it requires sample-collection and culture under a controlled environment. Moreover, the time required for culture makes the information obsolete by the time it is obtained. This method can never indicate the current bioaerosol load and this is a particular negative if the air quality is so bad that the adverse impact on the health of users of the space during the waiting time is difficult to remedy. In contrast, ATP bioluminescence techniques are simple, rapid, and cost-effective and allow monitoring of the spatial distribution of bioaerosol and variation in microbial activity in an indoor space. Microbial activity is defined here as the active microbial load in the sample, measured as RLUs (Chu *et al.*, 2001; Tiquia *et al.*, 2002; Horiuchi *et al.*, 2003).

In this study an ATP bioluminescence technique is used to measure indoor air microbial activity. The ATP bioluminescence results are compared with results obtained using the traditional culture method for enumeration of airborne microorganisms in order to confirm correlations between the ATP bioluminescence and traditional culture methodologies. Additionally, the feasibility of using an ATP bioluminescence technique for monitoring the efficiency of indoor disinfection is evaluated.

EXPERIMENTAL

Monitoring of Microbial Activity

In this study, the bioluminescence-based ATP hygiene monitoring system (SystemSURE II, Hygiena, UK) was used for monitoring microbial activity. The instrument was continuously calibrated to ensure its normal operation and accuracy. Bioaerosol was collected by sucking indoor air into an SKC QuickTake 30 sampler at 28.3 L/m^3 for 8 minutes. The bioaerosols deposited to aseptic cotton swabs, designed in our laboratory, in the sampler. An ATP-bioenzyme, D-luciferin, was then added to the sampler to react with the deposited bioaerosols and convert the bioaerosol ATP into AMP (Adenosine monophosphate). Bioluminescence was produced as shown by the following reaction, catalyzed by luciferase embedded in the kit:

$$\text{ATP} + \text{luciferase} + \text{D-luciferin (pigment)} + O_2 \rightarrow \text{Oxyluciferin} + \text{Luciferase} + \text{AMP} + \text{PPi} + CO_2 + \text{Light (560 nm)}$$

where PPi is inorganic pyrophosphate. The bioluminescence was measured with the bioluminescence instrument, which measures the bioluminescence as relative light units. ATP represents a source of energy released from all living cells, and we measured ATP in Relative Light Units (RLUs) as a proxy for microbial activity. The measured RLUs indirectly indicate microbial activity and thereby the quantity of microorganisms present. In theory, the relationship between measured ATP and quantity of microorganism should be perfectly linear. In practice, this relationship could be interfered with by variation in the age of microorganisms and scale of colonies. Simplifying the microbial activity, the relationship between ATP and RLU could be identified as 1 RLU $\fallingdotseq 1 \times 10^{-15}$ mol ATP.

Traditional Estimation of Bioaerosol

For comparison and validation, the traditional culture method was also used to collect bioaerosols. Bioaerosol was continuously collected using an SKC QuickTake 30 sampler. To ensure that the microbial counts were in a countable range after culturing, three different sampling volumes were collected by switching on the sampler air pump for 2, 5 and 8 minutes, at a sampling rate of 28.3 L/min, for a set of three samples. This eliminated the problem of too numerous to count (TNTC). Triplicate samples were analyzed to ensure the reliability of the results.

The concentration of total airborne microorganisms was calculated according to the reference method, NIEA E301.11C, published by the Taiwan Environmental Protection Administration. This reference method, NIEA E301.11C, is an amalgamation of other relevant reference methods (American Conference of Governmental Industrial Hygienists, 1989, 1999; Hung *et al.*, 2005; Yang and Heinsohn, 2007; Hsu *et al.*, 2012). The sampler forces air to directly impact a tryptic soy agar growth medium. Each liter of the agar medium contains 5.0 g NaCl, 15.0 g tryptone and 5.0 g soytone, thiopeptone or thiotone, and 15.0 g agar. Additionally, cycloheximide (100 μg/mL) was added for

inhibiting fungal growth. Additionally, the average relative humidity in these indoor environments ranged from 61% to 72% and such a relative humidity is not a favorable condition for mass propagation of fungal growth. The agar plate was then incubated at 30 ± 1°C for 48 ± 2 hrs, the total number of bacterial colonies was counted, and the final result was expressed in CFU/m^3 (colony forming units per cubic meter). A QuickTake 30 sampler was used to collect samples after which CFUs were observed, calibrated and calculated statistically by applying the positive hole correction conversion to the data. The magnitude of the positive hole correction and coincidence correction, however, increases exponentially with increasing colony counts. The application was usefully employed for every run. Meanwhile, the real environmental conditions are complicated with human activities and sampling limitations.

Monitoring Sites and Related Monitoring Devices

The research was conducted at four sites; a large Chinese medicine diagnostic room, a library, a government office, and a railway station lobby. The *walk through* method was applied and the route was determined based on the spatial characteristics of each site. When sampling locations at each site were selected, on-site measurements of various in-door air pollutants such as CO_2, CO, O_3, TVOCs (total volatile organic compounds), HCHO (formaldehyde), PM_{10} and $PM_{2.5}$ were also conducted. As noted in Table 1, an NDIR (non-dispersive infra-red) sensor was used for monitoring CO_2 and a PID (photo-ionization detector) was used for monitoring TVOC. Scattered laser light was used for monitoring PM_{10} and $PM_{2.5}$ and their equivalent mass concentrations were evaluated using a proprietary algorithm. Other species such as CO, O_3 and HCHO were detected using electrochemical methods. Table 1 also shows the measuring range, accuracy and resolution of the various monitoring devices. All equipment was calibrated according to specifications.

A Varimax-rotated principal component analysis (PCA) was performed to determine the principal component loadings of indoor air pollutants, temperature, humidity and microbial activity for all four sites.

RESULTS AND DISCUSSION

Comparison of Indoor Air Microbial Activity and Total Microbial Count

Conventional bacterial culture results, obtained using 17 air sample sets, which were collected using an SKC QuickTake 30 sampler, are shown in Fig. 1. The microbial numbers at some sampling points varied dramatically, revealing that this method can lead to significant differences in the number of microorganisms detected even within a very short period of time. Two sample sets contained average microbial numbers higher than the maximum level suggested by the World Health Organization (WHO) of 1000 CFU/m^3.

Bioluminescence intensity is a measure of the number of bioaerosol cells. The data obtained via ATP bioluminescence was compared with that obtained via the culture method and the results are shown in Fig. 2. Bioaerosol for comparison

Table 1. Specification of field-portable and direct-reading devices for indoor air quality monitoring.

	CO_2	CO	O_3	HCHO	TVOC	$PM_{10}/PM_{2.5}$	Microbial activity
Model	AirBoxx, KD Engineering	AirBoxx, KD Engineering	AirBoxx, KD Engineering	Formaldcmcter htV, PPM	PGM-30, RAE	Acrocct 531, Mct One	SystcmSURE II, Hygicna
Sensor	Referenced non-dispersive infra-red (NDIR)	Electrochemical	Electrochemical	Electrochemical	Photoionization detector (PID)	Double beam laser	Bioluminescence
Measuring range	0–10,000 ppm	0–500 ppm	0–2 ppm	0–10 ppm	0–2000 ppm	0–1 mg/m^3	0–9999 Relative light unit (RLU)
Accuracy	± 5% of reading	± 3% of reading	0.04 ppm	94% at 0.3 ppm level	± 10% of reading	± 10% to calibration aerosol	8–18%[a]
Resolution	1 ppm	0.1 ppm	0.001 ppm	0.01 ppm	0.1 ppm	0.001 mg/m^3	1 RLU ≒ Femtomoles of ATP
Sampling method	Diffusion	Diffusion	Diffusion	Snatch-sample of air	Diffusion	A constant airflow	Ultrasnap
Instrument read time	< 60 sec	< 45 sec	< 150 sec	< 10 sec	< 10 sec	< 120 sec[b]	< 15 sec

[a] Reproducibility or coefficient of variation was calculated using data from 10 replicates for each serial dilution of ATP pipetted onto swab tips from 2–2000 femtomoles.
[b] Sample time for mass mode.

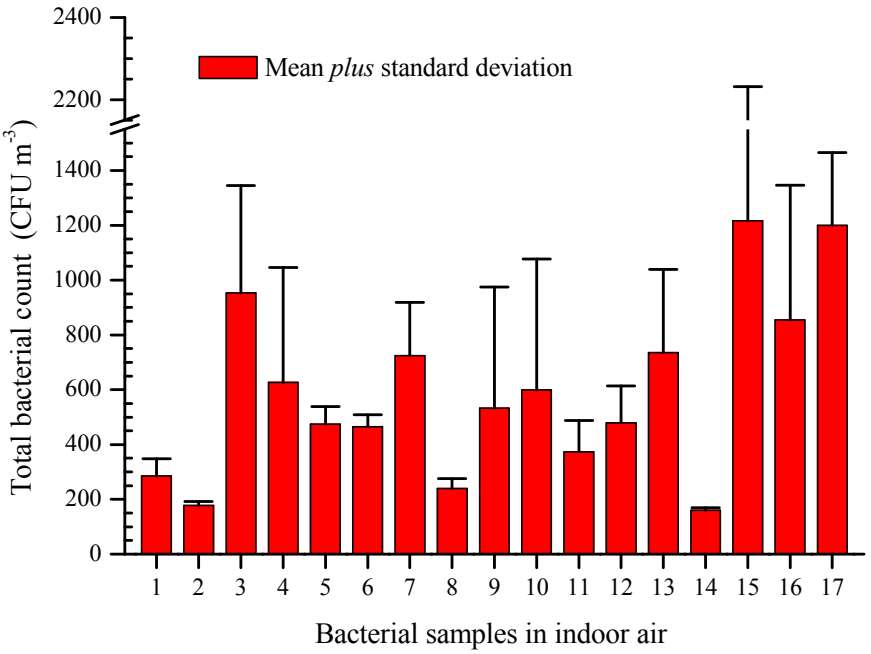

Fig. 1. Mean (plus standard deviation) values of bacterial samples in indoor air cultured by the traditional microbiological method.

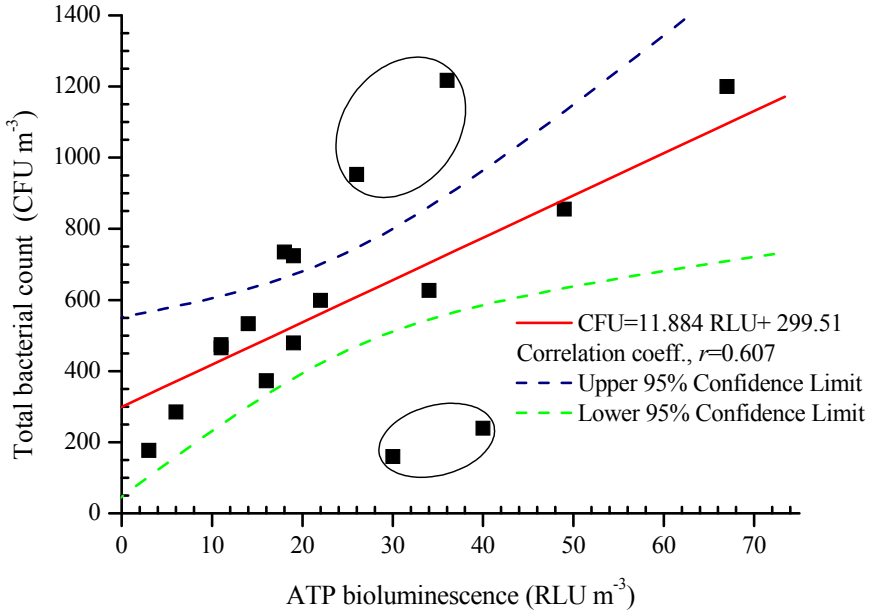

Fig. 2. Relationship between results of ATP bioluminescence and conventional culture-based culture method in indoor air.

of ATP bioluminescence and the traditional culture method was collected using an SKC QuickTake 30 sampler. The correlation coefficient (r) between total ATP bioluminescence microbial activity and the total microbial concentration obtained via culture was 0.607, which represents moderately high correlation. These two methods provide information in different ways yet, in general, the results on microbial activity obtained using ATP bioluminescence correlate with the bioaerosol concentrations as represented by CFUs. However, some individual sample comparisons show extreme differences. For example, two sets of samples have 26 RLU/m^3 and 36 RLU/m^3 of bioluminescence corresponding to incubated bioaerosol concentrations of 53 CFU/m^3 and 1217 CFU/m^3, respectively, and another two have 49 RLU/m^3 of bioluminescence to 855 CFU/m^3 and 67 RLU/m^3 to 1200 CFU/m^3. These differences in correlation between bioluminescence and culture results for some samples are mainly due to differences in the sampling methods. Different species of microorganisms may experience different collecting efficiencies, and the collected microorganisms may be damaged or killed due to violent impaction or dehydration. Two samples have a particularly low CFU count

compared to ATP bioluminescence, i.e., 30 RLU/m^3 versus 160 CFU/m^3, and 40 RLU/m^3 versus 239 CFU/m^3 and this may be because ATP bioluminescence is capable of detecting the existence of all viable microorganisms including bacteria, fungi and pathogens whereas the conventional culture-based method only enumerates the microorganisms that survive collection and culture. Indeed, the results obtained in this study (Fig. 1) and reported in the literature (Chen, 1997; Li *et al.*, 1999; Feng *et al.*, 2007; Hsu *et al.*, 2010) show that significant differences in the microorganism concentration exist for samples collected and analyzed under similar conditions using the culture method. A closer inspection of the data in this study reveals that six RLU-CFU data sets were outside the 95% confidence limit (Fig. 2) and three of these have relatively large standard deviations on total bacterial count, indicating significant variation in these counts. The totals of these six CFU counts do not correspond to their ATP bioluminescence values and if these sets are excluded, the remaining eleven sets of RLU-CFU data show high correlation between CFU and RLU values (CFU = 13.716 × RLU + 237.32; $r = 0.963$; p *value* < 0.001). These observations reveal that variations in different environmental factors may influence the correlation between results of the two methods. Nevertheless, the moderately high correlation obtained in this study for the overall results and the high correlation obtained with outlying CFU counts eliminated confirms that ATP bioluminescence can be used as a valuable reference for evaluating the potential of bioaerosols contained in indoor air.

Indoor Air Quality at Four Sites

ATP bioluminescence was used to evaluate microbial activity at four sites, i.e., a Chinese medicine diagnostic room, a library, a government office, and a railway station lobby. Nine other air quality parameters, CO_2, CO, O_3, HCHO, TVOC, $PM_{2.5}$, PM_{10}, temperature and relative humidity, were also monitored. Table 2 shows the results of these investigations. At the time of monitoring air quality, acupuncture and moxibustion acupuncture-therapy was being practiced in the Chinese medicine diagnostic room and this explains higher CO_2, CO, and HCHO levels than at the other three sites. Average PM concentrations in the Chinese medicine diagnostic room were higher than in the library and government office but lower than in the railway station lobby. However, maximum $PM_{2.5}$ and PM_{10} levels were highest in the Chinese medicine diagnostic room, at 124 and 180 μg/m^3 respectively. These highs exceed the suggested maximum $PM_{2.5}$ of 100 μg/m^3 and maximum PM_{10} of 150 μg/m^3. Indoor air CO_2 concentrations in the library, government office and railway station varied between 410 and 570 ppm indicating that these 3 sites have good ventilation. Unlike the diagnostic room, which is within a hospital building, all three of these sites are adjacent to the street, and the frequent ventilation draws the outdoor atmospheric CO emitted by passing vehicles into the room, resulting in higher observed indoor CO concentrations. HCHO levels were higher in the diagnostic room than at the other sites. The average HCHO concentration (0.15 ppm) in the Chinese medicine diagnostic room was two to three

Table 2. Air quality statistics at four indoor sites.

	CO_2 (ppm)	CO (ppm)	O_3 (ppm)	HCHO (ppm)	TVOC (ppm)	$PM_{2.5}$ (μg/m^3)	PM_{10} (μg/m^3)	Temp (°C)	RH (%)	Microbial activity (RLU/m^3)
Chinese medicine diagnostic room (n = 15)										
Min–Max	608–838	1.4–4.2	< 0.001–0.054	0.08–0.28	< 0.1	16–124	37–180	23.4–26.5	58–68	1–59
Mean	719	2.3	0.022	0.15	N.D.	35	66	24.8	63	20
Library (n = 12)										
Min–Max	410–569	1.3–2.6	< 0.001–0.031	< 0.01–0.08	< 0.1	11–21	40–70	23.8–25.2	59–63	14–41
Mean	505	1.7	0.017	0.06	N.D.	14	51	24.7	61	27
Government office (n = 13)										
Min–Max	418–555	0.6–0.9	< 0.001–0.019	0.02–0.05	< 0.1	15–28	39–95	22.4–24.7	62–68	3–41
Mean	482	0.8	0.011	0.03	N.D.	22	62	23.8	64	14
Railway station lobby (n = 8)										
Min–Max	420–505	1.0–1.6	< 0.001–0.026	0.02–0.09	< 0.1	35–53	71–122	20.5–21.8	70–75	70–157
Mean	457	1.3	0.017	0.05	ND	41	89	21.4	72	111

times higher than at the other sites, and higher than the critical HCHO concentration of 0.1 ppm in Taiwan. As noted, the therapeutic moxibustion process contributes to higher HCHO and PM concentrations (Mai, 2009), but poor ventilation is also a factor. The second highest average HCHO concentration (0.06 ppm) occurred in the library and the main cause of this is seen as volatile aldehydes produced during degradation of paper-based materials (Fenech *et al.*, 2010).

A railway station lobby is busy with human traffic, and hence experiences multiple sources of biological contamination. Reflecting this, its average microbial activity potential of 111 RLU/m^3 and wide range of 70 to 157 RLU/m^3 was far higher than at the other three sites. Average ATP bioluminescence at the government office was 14 RLU/m^3, at the library 27 RLU/m^3, and at the diagnostic room 20 RLU/m^3. In the latter, because air purification units are installed in the Chinese medicine diagnostic room, and the hospital emphasizes and practices sanitation and disinfection, its higher HCHO levels may inhibit or lower the indoor potential of microbial activity. The indoor air TVOC at all four sites was below detectable limits (0.1 ppm). Additionally, average indoor air O_3 was lower than atmospheric O_3 concentration (Tsai *et al.*, 2003; Tsai, 2005; Tsai *et al.*, 2007) at all four sites, indicating that there were no apparent sources of O_3 near these sites.

Correlation between Indoor Pollutants and Environmental Factors

The PCA aimed to determine the principal component loadings of indoor air pollutants, temperature, humidity and microbial activity for all four sites, and results are shown in Table 3.

For the Chinese medicine diagnostic room, three principal components (PCs) produced eigenvalues greater than 1.0, together representing 85.47% of the total explained variance. In PC1, correlation loadings were 0.78, 0.97, and 0.99 for CO, $PM_{2.5}$ and PM_{10}, respectively. These observations indicate that the occurrence of PM in the Chinese medicine diagnostic room is closely related to incomplete combustion from the therapeutic moxibustion acupuncture performed in the room. In PC2, the correlation loadings were 0.88 and 0.88 for CO_2 and HCHO, indicating that HCHO is a result of the moxibustion. Additionally, high negative correlations between temperature and relative humidity and between temperature and RLUs shows that the microbial activity in the Chinese medicine diagnostic room is influenced by environmental factors; lower microbial activity is associated with higher temperature and lower RH.

For the library, three PCs produced eigenvalues greater than 1.0, together representing 84.88% of the total explained variance. In PC1, O_3, $PM_{2.5}$ and PM_{10} showed high positive correlation that is closely related to the high ventilation rate drawing a large quantity of outdoor air into the room. The small quantity of HCHO emitted by books and finishing materials (Fenech *et al.*, 2010) is thus diluted so that is has a high negative correlation with the above three parameters. Additionally, the HVAC (heating, ventilation and air conditioning) system that lowers the library indoor temperature brings outdoor atmospheric air into the library and this air is rich in traffic-related emissions, i.e. CO, and CO_2. Hence, the library air shows high correlations among room temperature, CO and CO_2 in PC2. Moreover, the RLU value is a strong negative loading variable in PC3, indicating that the library indoor microbial activity and the monitored air pollutants do not originate from the same source.

For the government office, two PCs produced eigenvalues greater than 1.0, together representing 70.25% of the total explained variance. In PC1, CO_2 shows high correlation loadings with $PM_{2.5}$ and PM_{10} indicating a similar source for these pollutants. In PC2, temperature and RH have correlation loadings of as high as 0.94 and –0.97, respectively; thus these two environmental factors have high negative correlation. Microbial activity does not show any obvious correlation with room temperature and relative humidity in the government office.

Table 3. Varimax-rotated principal component loadings of indoor air pollutants, temperature, humidity and microbial activity (RLU) at four places.

	Chinese medicine diagnostic room			Library			Government office		Railway station lobby	
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC1	PC2
CO_2	0.19	**0.88**	0.03	0.60	**–0.70**	–0.18	**–0.91**	0.04	**0.92**	0.04
CO	**0.78**[a]	0.14	0.59	–0.13	**–0.81**	–0.44	0.66	–0.62	**–0.70**	–0.39
O_3	–0.15	–0.48	–0.33	**0.92**	0.11	0.03	–0.62	0.24	0.37	–0.47
HCHO	–0.12	**0.88**	0.03	**–0.92**	–0.02	–0.01	–0.64	–0.35	**0.96**	–0.14
$PM_{2.5}$	**0.97**	0.09	0.17	**0.95**	0.03	–0.28	**0.92**	–0.14	**0.95**	–0.09
PM_{10}	**0.99**	0.03	0.08	**0.83**	0.09	0.23	**0.90**	–0.04	**0.99**	0.01
Temperature	0.25	0.09	**0.95**	–0.08	**–0.92**	0.29	0.01	**–0.97**	**–0.94**	0.16
Relative humidity	–0.20	0.07	**–0.97**	0.46	0.66	–0.48	0.05	**0.94**	**0.97**	–0.15
RLU	–0.02	–0.44	**–0.82**	0.05	–0.01	**0.83**	0.38	0.18	0.11	**0.92**
Eigenvalue	4.30	1.87	1.52	3.96	2.36	1.32	3.99	2.33	6.14	1.28
Cumul. Eigenvalue	4.30	6.17	7.69	3.96	6.32	7.64	3.99	6.32	6.14	7.42
% variance	47.80	20.76	16.92	43.99	26.24	14.65	44.33	25.92	68.25	14.19
Cumul. % variance	47.80	68.56	85.47	43.99	70.23	84.88	44.33	70.25	68.25	82.44

[a] Bold marked component loadings were ≥ |0.70|, indicating a significant component loading.

In the railway station lobby, two PCs produced eigenvalues greater than 1.0, together representing 82.44% of the total explained variance. In PC1, all variables except O_3 and microbial activity have high correlation loadings. This indicates that in the semi-open railway station lobby, the indoor air quality is significantly affected by outdoor atmospheric pollutants by direct airflow from outside and also from the HVAC system and frequent human traffic. Hence, both indoor and outdoor pollutants affect air quality in the railway station lobby. In PC2, microbial activity is the only variable that has correlation loading, but this is uncorrelated with other variables. This indicates that microbial activity and the indoor pollutants identified do not originate from the same sources, and hence that the relatively high microbial activity potential in the railway station lobby may be an independent feature of this site, linked to the throughput and density of people.

PCA correlations show that only in the diagnostic room is there significant correlation between RLUs and the room temperature or humidity. Lowering humidity of the Chinese medicine diagnostic room can reduce the indoor microbial activity potential. At all four sites, the indoor microbial activity potential does not originate from the same sources as the indoor air pollutants.

Spatial Distribution of Indoor Microbial Activity

The spatial microbial activity data collected during walk through of the four sites are shown in Fig. 3. It can be seen (Fig. 3(a)) that in the diagnostic room the entrance has the most microbial activity. This may be due to human traffic immediately outside the diagnostic room where the hospital lobby is located, indicating that the diagnostic room itself is relatively free of microbial activity. Also, there is a lesser but perceptible rise in microbial activity in a corner of the room adjacent to an exhaust fan. In the library (Fig. 3(b)), three regions, i.e., a storage area ('hovel'), an area centered on shoe racks, and an area centered on a plant but encompassing the library entrance, have relatively higher microbial activity, indicating that the on-site furniture arrangement affects the pattern of microbial activity. In the government office, contour lines (Fig. 3(c)) show higher microbial activity in the region located between the service desk and office desk, but once again 'furniture', i.e., indoor plants and a waste basket, appears to be the major source of microbial activity. Microbial activity in the railway station lobby (Fig. 3(d)) was higher than at the other three sites. Within the lobby, the highest microbial activity was at the main entrance (noted as two exits on the left of the diagram in Fig. 3(d)) and in the waiting area. In the absence.

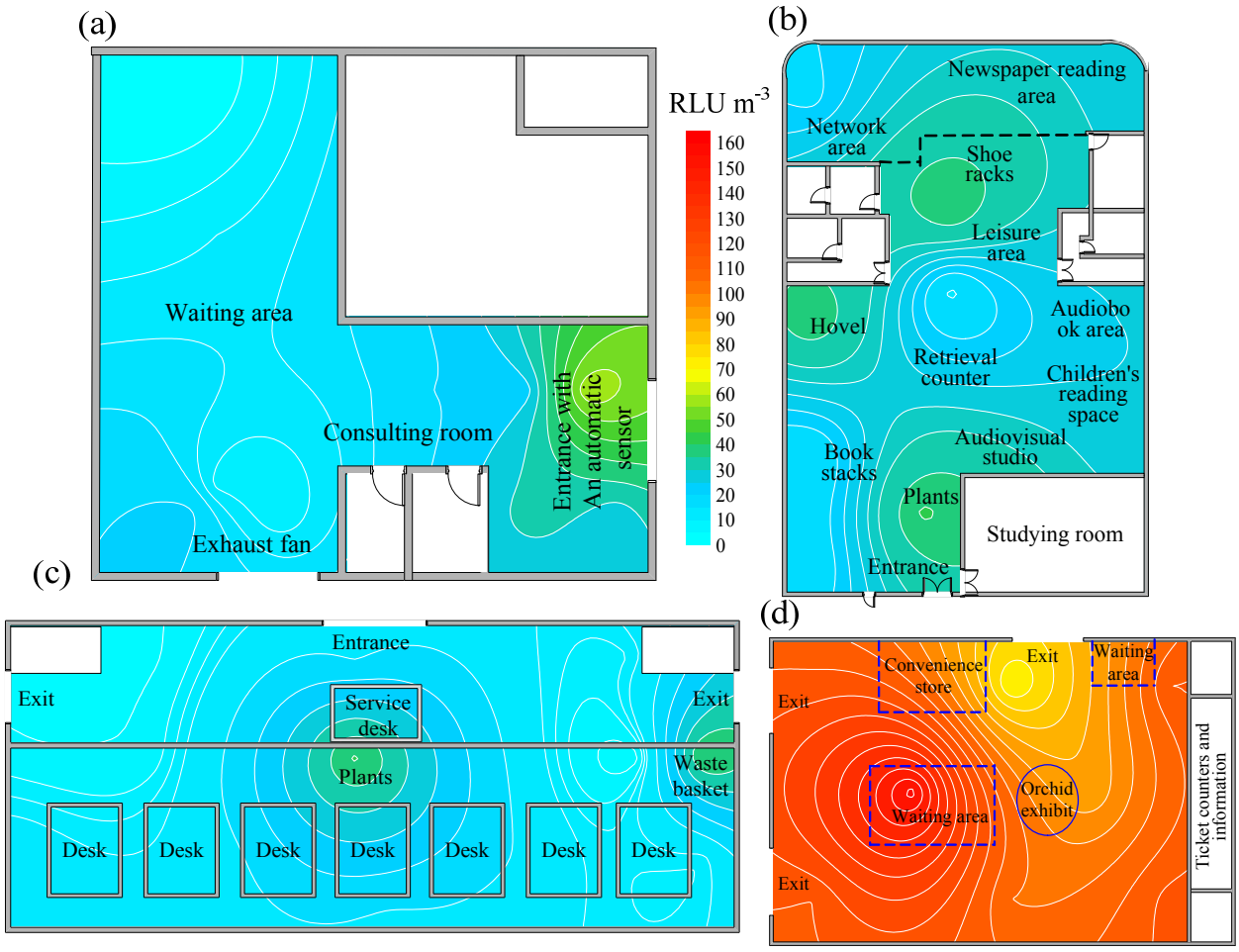

Fig. 3. RLU contour maps of spatial ATP bioluminescence at four indoor sites. (a) Chinese medicine diagnostic room, (b) Library, (c) Government office, and (d) Railway station lobby.

of other sources extant in the lobby, it is likely therefore that microbial activity is related to number and density of people. Bioaerosol concentration can also increase as a result of physical contact between people and between people and chairs in the waiting area

ATP Bioluminescence as a Tool for Monitoring Disinfection Efficiency

HVAC system, UV irradiation, electric ion emissions, iodinated biocidal filter media and nano metal-coating media are the available control methods to achieve acceptable biological removal/disinfection efficiencies with highly variable origins in indoor air (Lin and Li, 2002; Li and Wen, 2003; Ratnesar-Shumate *et al.*, 2008; Kwon *et al.*, 2011; Lee, 2011; Cheng *et al.*, 2012). Chloride dioxide (ClO_2), which is recommended as a safe disinfectant by WHO, has also been widely applied for disinfecting indoor air to eliminate bioaerosols. Hsu *et al.* (2010, 2012) demonstrated that multiple and regular ClO_2 applications yielded 4–8 h disinfection efficiencies of more than 59.0% in a local student health center in Taiwan; in steam room effective disinfection of 96.5% by spraying 107 mL of ClO_2 solution (250 mg/L) into the space of 90 m with an average number of persons of 20 in the room. The data analysis also yielded a disinfection time constant of the order of 10 minutes, which implies chlorine dioxide is a relatively fast acting disinfectant (Ling *et al.*, 2008). In this research, the ATP bioluminescence technique was tested as a rapid monitoring method for evaluating indoor air disinfection efficiency. A ClO_2 solution of 100 ppm was used as the disinfectant to conduct semi-open space disinfection. Airborne microbial activity was monitored 20 minutes before and 20 minutes after the disinfectant was applied and results (Fig. 4) indicated 61.3% disinfection efficiency. In addition to confirming the disinfection efficiency of ClO_2, the results show that the ATP bioluminescence method used here provides a rapid and effective tool for monitoring indoor air microbial quality, and hence one that is valuable for evaluating indoor air disinfection efficiency.

SUMMARY AND CONCLUSIONS

In this research, an ATP bioluminescence method is proposed for evaluating the distribution of bioaerosol activity potential in indoor air. The proposed method was compared with a traditional culture method and it was found that RLUs as a representation of bioaerosol activity had a moderately high correlation with CFU counts obtained using the conventional culture-based method (r = 0.607). Moreover, after removing outlying CFU counts from the calculation, the correlation rose to 0.963 (p value < 0.001), confirming the efficacy of the bioluminescence method. Four indoor sites were selected for study; a Chinese medicine diagnostic room, a library, a government office, and a railway station lobby. Air quality assessments showed that PM and HCHO levels were higher in the Chinese medicine diagnostic room and this was attributed to the use of moxibustion acupuncture in the room. HCHO may restrain microbial growth and propagation. ATP bioluminescence data for the four sites showed that microbial activity was much higher at the railway station lobby than at the other three sites and this was attributed to the greater volume of human traffic. Contrary to the general belief that hospital indoor air has relatively high microbial activity, RLUs showed that activity was generally equivalent to the air in the government office and in the library. Therefore, in the Chinese medicine diagnostic room lower ATP bioluminescence may be partly attributed to higher HCHO concentrations. PCA revealed that microbial activity in the Chinese medicine diagnostic room was correlated with room temperature and humidity and hence lowering these can decrease microbial activity. At all four sites PCA identified

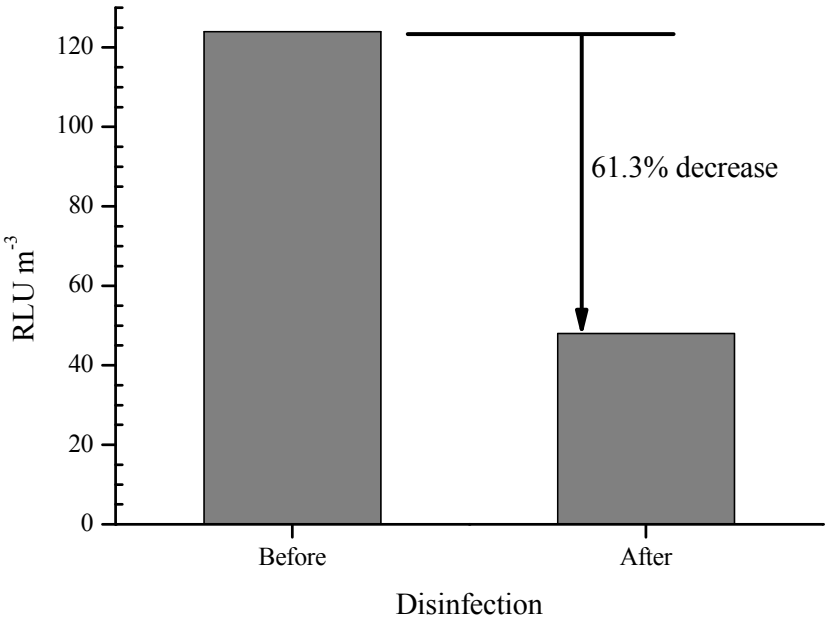

Fig. 4. Indoor spatial ATP bioluminescence 20 min before and after 100 ppm ClO_2 disinfection.

no source relationship between microbial activity and air quality parameters. Spaces near indoor plants, shoe racks and waste baskets were seen to have relatively higher microbial activity potential. The proposed ATP bioluminescence method was applied for the rapid evaluation of chloride dioxide disinfection efficiency and results confirmed that the method was effective. ATP bioluminescence is therefore a cost-effective and rapid tool for monitoring indoor air bioaerosols and providing indoor microbial spatial distribution data.

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