



Investigation and disinfection of bacteria and fungi in sports fitness center

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Received: 18 February 2021 / Accepted: 3 May 2021 / Published online: 20 May 2021

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Abstract

This study investigated the air quality improvement in terms of bacterial and fungal contamination in an exercise room of a fitness center under normal operating conditions. Environmental conditions including air conditioning, ventilation, moisture, CO₂, particulate matters, and total number of users were also recorded. In addition, fungal and bacterial load were assessed and disinfection on sports equipment surface was also examined. Background bacteria and fungi densities in bioaerosols were in the range of 249 ± 65 to 812 ± 111 CFU/m³ and 226 ± 39 to 837 ± 838 CFU/m³ in the exercise room of the fitness center and 370 ± 86 to 953 ± 136 CFU/m³ and 465 ± 108 to 1734 ± 580 CFU/m³ in the outdoor air, respectively. Chlorine dioxide and weak acid hypochlorous water aerosols could remove both bacteria and fungi much better than water scrubbing. Contact time of 15 min was sufficient to control both bacteria and fungi to comply with the official air quality standards. User density and carbon dioxide deteriorated both bacteria and fungi disinfection performance whereas temperature was only statistically significant on fungi disinfection. Other factors including relative humidity, airflow velocity, and particulate matters did not have any statistically significant effect on microbial inactivation. Apart from bioaerosol disinfection, inactivation of microorganisms on surfaces of sports equipment was also conducted using chlorine dioxide, zinc oxide, weak acid hypochlorous water, and commercial disinfectant. The surfaces of bicycle handle, dumbbell, and sit-up bench were found to be contaminated with bacteria. Overall bacterial load was 390 to 3720 CFU/cm² with *Escherichia coli* specifically 550 to 1080 CFU/cm². Chlorine dioxide and zinc oxide were noticeably better than weak acid hypochlorous water and commercial disinfectant in terms of bacteria inactivation whereas all tested disinfectants had comparable effectiveness on *E. coli* disinfection. Targeted microorganisms on the sports equipment surface were sufficiently inactivated within 2 min after the application of disinfectant.

Keywords Fitness center · Bioaerosols · Disinfectant

Responsible Editor: Diane Purchase

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Introduction

The current physical inactivity pandemic is one of the major leading risk factors for death worldwide. The World Health Organization (WHO) recommends at least 150 min of moderate-intensity physical activity throughout the week (Ramos et al. 2015; WHO 2018) for improving health benefits and overall well-being, such as walking, cycling, or sports. However, urban parks or indoor public spaces particularly in large cities usually have poor air quality due to traffic-related pollution. As a result, people who are living in urban areas prefer to work out in an indoor fitness center to avoid air pollution. Nonetheless, despite new recommendations of indoor air quality expanded to indoor public spaces, adoption has been slow. It largely depends on building construction, building materials, and type of ventilation together with

environmental factors such as natural ventilation, humidity, and temperature (Saini et al. 2020). In addition, number of users and fitness activities also play an important role in the air quality inside the fitness center.

Bacteria and fungi can pose significant risk to human health under fitness center environments. The main source of bacteria present on the surface of fitness facilities is people who come to exercise (Ramos et al. 2014). Dalman et al. (2019) found 38.2% of the environmental surfaces in 16 fitness facilities in Northeast Ohio in the USA were contaminated with *Staphylococcus aureus*, a common gram-positive bacterium species found in the nose and throat of humans. On the other hand, most fungal sources are located outdoors, e.g., soil, plants, and water bodies. However, the spores can readily penetrate indoor environments by means of human transport and ventilation systems.

The Environmental Protection Administration (EPA) of Taiwan has stated that, for indoor public spaces, the bacteria concentration should be not higher than 1500 CFU/m³, while the fungi concentration should not exceed 1000 CFU/m³ (EPA Taiwan 2014). Unfortunately, Taiwan lies in a subtropical zone where the climate is generally warm and humid all year round. This type of climate is highly conducive to the breeding and spreading of bioaerosols (Tsai and Liu 2009). Moreover, recent spread of COVID-19 worldwide shows that drinking water disinfection (Garcia-Avila et al. 2021) and indoor air purification are extremely important and necessary.

Various types of chemical disinfectants can be used against microorganisms including glutaraldehyde, formaldehyde, and chlorhexidine (McDonnell and Russell 1999). However, the use of such disinfectants—though effective in industrial setting—may be harmful when used in public application. Thus, the toxic, corrosive, and volatile properties must be thoroughly evaluated.

Chlorine dioxide (ClO₂) can be used as an oxidizing agent or disinfectant. It is a very powerful oxidizer that effectively eliminates pathogenic microorganisms including fungi, bacteria, and viruses. As a disinfectant and pesticide, it is mainly applied in a liquid form (Lenntech B.V. 2020). Weak acid hypochlorous water (WAHW), a most widely used disinfectant with good antimicrobial activity, can also be used as well (Chen et al. 2012; Nguyen et al. 2021). WAHW is environmentally friendly since it is unaffected by water hardness (Rutala and Weber 1997). Likewise, antibacterial activity of zinc oxide (ZnO) nano-powders has received significant interest worldwide (Sirelkhatim et al. 2015; Dimapilis et al. 2018).

In this study, ClO₂ and WAHW, which are applicable for air purification, were used to determine their microbicidal disinfection efficiency on bioaerosols present in the fitness center. Water was also used to serve as a control for scrubbing effect. The evaluation covers indoor air quality and sports equipment. The air inside the studied rooms of the fitness center was disinfected in a patron

concentration manner, i.e., 0, 5, and 10 patrons. Environmental factors including temperature, relative humidity, and airflow velocity were measured and maintained steadily. In all aerosol disinfection scenarios, the solution was applied using EP606 aerosol devices (GAO-SHUO Corporation, Taiwan) for 60 min after the exercise period. Bacteria and fungi remaining in the aerosols were periodically monitored and compared. For sports equipment study, three equipment with different surface characteristics were tested including bicycle handle with soft and foam-like surface, dumbbell with rough steel surface, and sit-up bench with smooth leather surface. Disinfectant solution of either ClO₂, WAHW, ZnO, or commercial disinfectant was used to clean all contact surfaces of each equipment after each of the exercise session. Total bacteria and *Escherichia coli* (*E. coli*) remaining on the surface were periodically monitored and compared. *E. coli*, a common gram-negative bacterium, was chosen as a representative species since several researchers have already investigated the gram-positive bacterium as a metric of microbial contamination in a fitness center and gymnasium (Ryan et al. 2011; Markley et al. 2012; Mukherjee et al. 2016; Dalman et al. 2019). The outcomes from this study will fulfill the overall perspective of bacterial contamination on sports equipment.

Materials and methods

This study was conducted in the fitness center at Chia-Nan University of Pharmacy and Science in Taiwan. This study consists of two parts: (1) determination of bacteria and fungi existence in the indoor air and (2) determination of bacteria and *E. coli* existence on the surface of sports equipment, both before and after treatment with various disinfectants. All experimental scenarios were replicated to ensure data reliability. The details of the experimental procedure are described in the sections below.

Study area

Microbial sampling was undertaken in a 332 m³ bicycle room in a fitness center of Chia Nan University of Pharmacy and Science in Tainan, Taiwan. Sampling was taken at six locations, including five locations inside and one location outside the bicycle room as shown in Fig. 1.

Disinfection method

Several disinfectants including ClO₂, WAHW, ZnO (5.4 wt% nanopowder aqueous solution with APS < 20 nm, LIWEI Nano Tech Co., Ltd.), and commercial disinfectant with cetrimide and chlorhexidine digluconate as the active

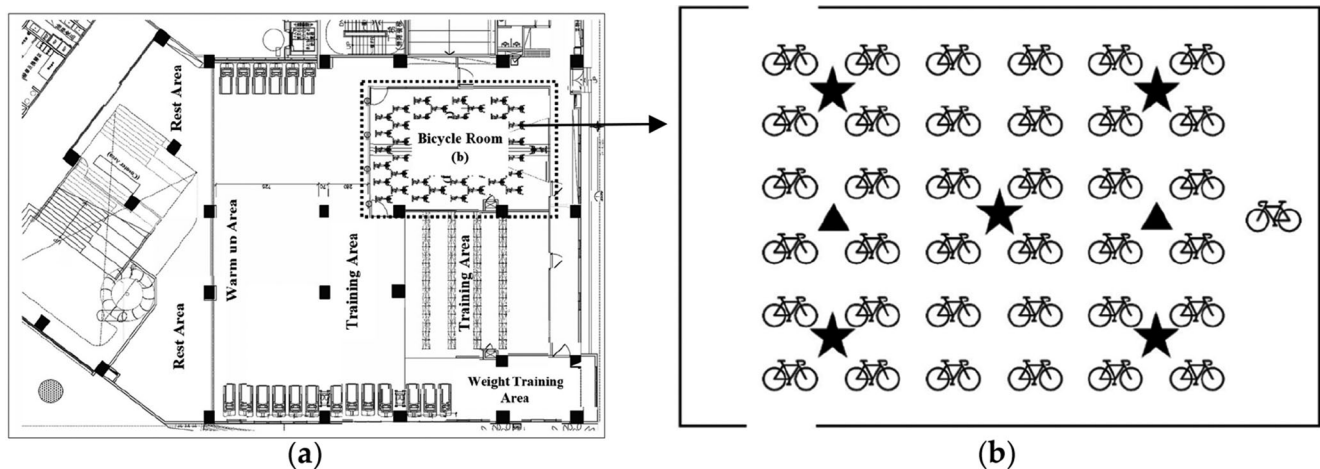


Fig. 1 Floor plans of sampling areas. (a) Total area. (b) Bicycle room. (🚲 = outdoor sampling location ★ = sample location; ▲ = disinfecting aerosol applying location)

ingredients (Savlon, ITC Limited) were applied in this study in order to disinfect or remove the microorganisms. For bioaerosol study, ClO_2 solution and WAHW were used. Control experiments with water (no disinfecting chemical) were conducted to investigate the effect of physical scrubbing. As described above, the bicycle room volume is 332 m^3 . To satisfy the 8-h time-weighted average (TWA) maximum exposure limit of 0.3 mg/m^3 for ClO_2 (OSHA 2020), ClO_2 disinfection in the bicycle room was performed using 400 mL ClO_2 solutions (at 250 mg/L). No maximum exposure limit is prescribed for WAHW disinfection. Nonetheless, in order to control the amount of aerosols being introduced into the studied boundary, the rates of WAHW (at 250 mg/L) and water aerosolization were similar to those of ClO_2 . The two EP606 aerosol devices (GAO-SHUO Corporation, Taiwan) placed at two different locations in the bicycle room were switched on just after the termination of all exercise activities, and all users had left the room and continued for 60 min.

For bacterial inactivation on the surface of sports equipment, ClO_2 , WAHW, ZnO, and commercial disinfectant (Savlon, ITC Limited) at the concentration of 50, 50, 400, and 400 mg/L were used. The surface of the sports equipment was wiped thoroughly by the sterilized cloth dampened with disinfectant mixture.

Sample collection

Air samples were collected in accordance with the NIEA (National Institute of Environmental Analysis) guidelines specified by the Taiwan Environmental Protection Administration (TEPA) (Taiwan EPA 2016a, b). On each sampling day, indoor air samples were collected before and after the exercise session (either with 5 and 10 users) at 15 and 60 min. The samples were collected using a MAS-100 Eco Microbial Air Sampler (Merck, Germany, @ 200 L/min) in

petri dishes filled with tryptic soy agar (TSA) plates and malt extract agar (MEA) for bacteria and fungi enumeration, respectively. Air flow velocity, temperature, relative humidity, and particulate matters (PM10, PM7, PM2.5, and PM1) were also measured.

Collection of microbial samples on sports equipment, i.e., bicycle handle, dumbbell, and sit-up bench, was performed before the cleaning and after cleaning at 2, 5, 10, and 30 min. Five random swabs were performed for each sports equipment. Each swab was done on 1-cm^2 surface of the sports equipment using a sterilized cotton bud soaked with basal medium. The swabbed cotton buds were then further processed for microbial enumeration. To determine the amount of viable microorganisms, the samples were cultivated on Petri dishes with plate count agar (PCA) plates for bacteria and chromocult coliform agar plates for *E. coli*.

Analytical methods

Measurement of environmental factors

The temperature, relative humidity, airflow velocity, and carbon dioxide concentration were evaluated by The VelociCalc® Multi-Function Ventilation Meter 9565 (TSI Inc., USA). Light meter pocket (Lutron Electronic, LX-103) was used to measure the illumination in the room. Particulate matter was measured by handheld particle mass counter (AEROCET 531, Met One Instruments Inc., USA).

Measurement of disinfectants concentration

The concentration of chlorine dioxide and hypochlorous acid was determined by using light absorbance measurement by waterproof portable colorimeter (OAKTON Instrument, USA).

Microbiological analysis

For the bioaerosol bacteria, the TSA plates were incubated at the temperature of 30 ± 1 °C for 48 ± 2 h. Meanwhile, the MEA plates were incubated at 25 ± 1 °C for 4 ± 1 day for measurement of bioaerosols fungi. The bacteria and fungi concentrations were then evaluated by counting the colonies formed on the respective agar surfaces.

For microorganism numeration from sports equipment, the initial sample was diluted with Butterfield's phosphate-buffered dilution water (PBS) at the dilution of 1:10, 1:100, 1:1000, and 1:10,000. After that, appropriate amount of the diluted sampled was dropped into the plate count agar (PCA) or chromocult coliform agar in the Petri dishes and then swirled the plate to homogeneously mix the sample and culture medium together. Allow the mixture to solidify and then turn the culture dish upside down. Then, the PCA plates were incubated at a temperature of 35 ± 1 °C for 48 ± 2 h. Meanwhile, the chromocult coliform agar plates were incubated at 35 ± 1 °C for 48 ± 2 h for the *E. coli* cultivation. Count the number of colonies on the culture plate and calculate total microbial count in terms of CFU/mL (Jaemsri and Rujak 2017).

Statistical analysis

The experimental data were analyzed statistically using a commercial SPSS software (IBM SPSS Statistics 22.0). The correlations of the bioaerosols disinfection effectiveness and environmental factors (i.e., the temperature, relative humidity, airflow velocity, carbon dioxide concentration, and particulate matters) were examined by means of Pearson's correlation analysis ($p < 0.05$).

Results and discussion

Bioaerosols in indoor air

Initial concentration of bioaerosols

The initial concentrations of bacteria and fungi before the disinfection treatment are shown in Table 1. Average temperature, relative humidity, airflow velocity, carbon dioxide, PM1, PM2.5, PM7, and PM10 during the experimental periods were 18.9 to 24.9 °C, 62.0 to 80.6%, 0.3 to 1.2 m/min, 398 to 1298 ppm, 0 to 1.12 mg/m³, 0.001 to 1.170 mg/m³, 0.002 to 1.175 mg/m³, and 0.002 to 1.179 mg/m³, respectively. The magnitude of these environmental factors represents the typical conditions of the indoor air in the fitness center. It is necessary to note that the experiments were conducted at existing conditions and different times; hence, the initial concentrations of bacteria and fungi in each experiment were

different. Therefore, the determination of whether an indoor environment is polluted or not is often based on a comparison of bioaerosol concentration in the indoor air with that of outdoors (Zhu et al. 2003). It can be seen that the indoor air tended to contain less bacteria and fungi than the outdoor air (outside the sport center); nonetheless, there was no specific correlation. This is possibly due to the bioaerosol filtration effect via air conditioning system of the fitness center and the activities happened prior to the tested session.

Effect of environmental factors on bioaerosols distribution

Table 2 shows the correlations between the bacteria and fungi colony counts and the environmental factors after the disinfection treatment process. Significant positive correlations were found between the effectiveness of bacteria disinfection and the effectiveness of fungi disinfection ($r = 0.610$, $p < 0.01$). By contrast, significant negative correlations were obtained between the effectiveness of bacteria disinfection and the number of users ($r = -0.584$, $p < 0.05$) and carbon dioxide concentration ($r = -0.581$, $p < 0.05$). Significant negative correlations also existed between the effectiveness of fungi disinfection and the number of users ($r = -0.690$, $p < 0.01$), temperature ($r = -0.517$, $p < 0.05$), and carbon dioxide concentration ($r = -0.680$, $p < 0.01$). In other words, the effectiveness of bacteria disinfection increased along with the effectiveness of fungi disinfection but decreased with an increasing number of users and carbon dioxide concentration. Similarly, the effectiveness of fungi disinfection decreased with an increasing number of users, temperature, and carbon dioxide concentration. Carbon dioxide level highly correlated to the user density with the p value of less than 0.01 which was expected because the major source of carbon dioxide in the fitness center was from human respiration. Other factors including relative humidity, airflow velocity, and particulate matters had no statistically significant impact on both bacteria and fungi inactivation. Nonetheless, it was found that air velocity and particulate matters (PM1, PM2.5, PM7, PM10) were related to one another at the 0.05 level. This should be primarily due to the particle filtration by air conditioning system. In addition, Table 2 also suggested that all particulate matters present in the air inside this fitness center are likely derived from the same source since all of them are highly correlated to one another at the significant level of 0.01.

Effectiveness of bioaerosols disinfection

The effectiveness of bacteria and fungi disinfection by three different methods: ClO₂, WAHW, and water were compared within treatment period of 15 and 60 min as shown in Figs. 2 and 3, respectively. The results showed that all the methods had the ability to reduce bacteria and fungi present in the

Table 1 Initial concentration of bacteria and fungi (mean \pm SD)

	Indoor			Outdoor		
	ClO ₂	WAHW	Water	ClO ₂	WAHW	Water
Initial concentration of bacteria (CFU/m ³)						
0 people	401 \pm 179	249 \pm 65	308 \pm 231	855 \pm 41	370 \pm 86	613 \pm 163
5 people	761 \pm 169	470 \pm 238	433 \pm 172	953 \pm 136	616 \pm 203	498 \pm 124
10 people	715 \pm 153	619 \pm 259	812 \pm 111	765 \pm 403	684 \pm 591	879 \pm 83
Initial concentration of fungi (CFU/m ³)						
0 people	226 \pm 39	228 \pm 53	618 \pm 499	1203 \pm 603	873 \pm 460	1064 \pm 139
5 people	837 \pm 838	275 \pm 212	307 \pm 16	1734 \pm 580	738 \pm 174	908 \pm 706
10 people	492 \pm 278	255 \pm 133	613 \pm 319	1220 \pm 156	465 \pm 108	764 \pm 677

Remark: "N" = 2 or 3

aerosols in the indoor air although water which served as a control had the lowest capability in both bacteria and fungi removals. Hence, it implies that the removal of bacteria and fungi in the presence of ClO₂ and WAHW was mainly derived from chemical interaction rather than physical phenomenon. The inactivation potential decreased as the number of users increased. It is interesting to observe that fungi can be inactivated in all treatment scenarios at 15 min of contact time but ClO₂ had the highest efficiency. However, the number of fungi noticeably rebounded in the WAHW and water scrubbing treatments at 60 min. This regrowth behavior of fungi might be due to the ability of fungi to form spores to protect themselves from inactivation. Another factor might be due to an increase in relative humidity after prolonged aerosolization which could promote the enumeration of fungi (Rajasekar and Balasubramanian 2011). Figures 2 and 3 also illustrate that

ClO₂ seemed to be a better fungicide whereas WAHW seemed to be a slightly better bactericide than ClO₂ for long time exposure.

According to Ishihara et al. (2017), the hypochlorous acid in aqueous solution is unstable against ultraviolet (UV) light, sunshine, air contact, and elevated temperature (≥ 25 °C); hence, ClO₂ seems to be a better choice for bioaerosol disinfection. As shown in Table 1, bacteria and fungi disinfection effectiveness were not related to treatment time; hence, disinfecting the fitness room for 15 min after usage is a more appropriate option that can reduce both treatment time and cost. Within the ranges obtained during the experimental period, other compositions in the room including temperature, relative humidity, air flow velocity, PM₁₀, PM₇, PM_{2.5}, and PM₁ had no statistically significant effect on bacteria and fungi disinfection.

Table 2 Correlations between disinfection effectiveness and environmental factors

	% effective on bacteria disinfection	% effective on fungi disinfection	No. of users	Time	Temp.	Relative humidity	Airflow velocity	Carbon dioxide	PM ₁	PM _{2.5}	PM ₇
% effective on fungi disinfection	0.610**										
No. of users	-0.584*	-0.690**									
Time	0.114	-0.302	0								
Temp.	-0.258	-0.517*	0.564*	-0.032							
Relative humidity	-0.026	-0.209	0.319	0.141	-0.138						
Airflow velocity	-0.222	-0.056	0.379	0.024	0.179	0.042					
Carbon dioxide	-0.581*	-0.680**	0.961**	0.058	0.411	0.483*	0.32				
PM ₁	-0.198	-0.249	0.39	-0.016	0.477*	0.293	0.518*	0.383			
PM _{2.5}	-0.07	-0.147	0.322	-0.015	0.433	0.334	0.522*	0.314	0.986**		
PM ₇	0.023	-0.072	0.267	-0.012	0.396	0.359	0.512*	0.259	0.959**	0.993**	
PM ₁₀	0.023	-0.077	0.273	-0.007	0.395	0.364	0.510*	0.268	0.959**	0.993**	1.000**

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

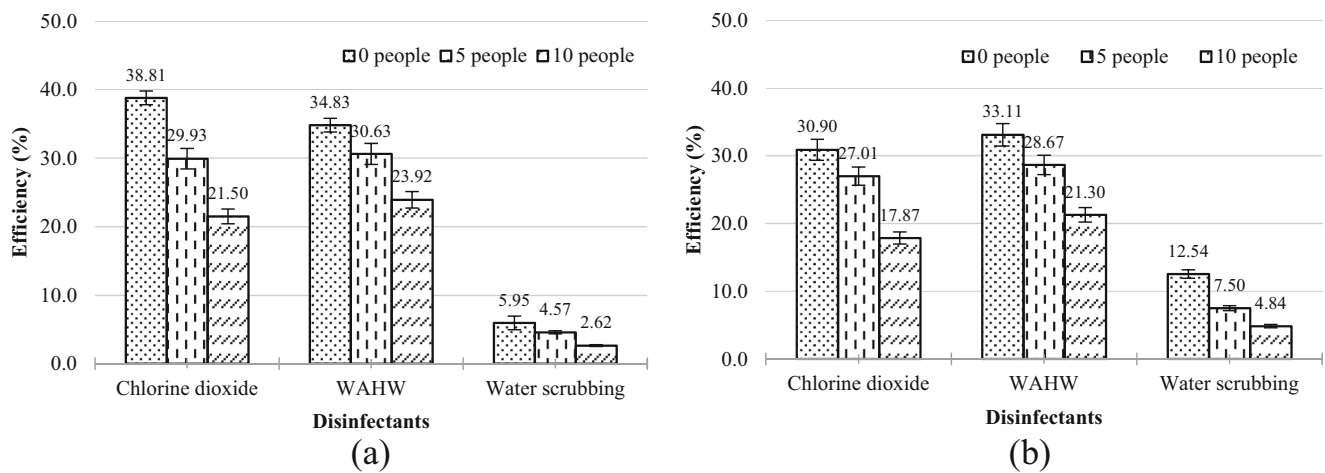


Fig. 2 Effectiveness of bacteria disinfection (a) after 15 min and (b) after 60 min (N = 2 or 3)

The results from this study showed that the effectiveness both of bacteria and fungi disinfection decreased with an increasing number of users, time, temperature, and carbon dioxide concentration. It was found that ClO₂ seemed to be the most appropriate selection for bacteria and fungi control in the bioaerosols under the studied conditions. This is because ClO₂ is a very powerful oxidizer that effectively eliminates pathogenic microorganisms including fungi, bacteria, and viruses (Lenntech B.V. 2020). Findings from this study are in agreement with several other studies using ClO₂ to control the amount of bioaerosols in indoor air including student cafeterias (Hsu et al. 2014), library (Hsu et al. 2015), and pet shop (Lu et al. 2018). These studies recommended that treatment with ClO₂ is an efficient way of compliance with the Taiwan EPA guidelines for indoor air quality. Nonetheless, this study found that airflow velocity had no significant effect on both bacteria and fungi removal which contrasts with the observations from Hsu et al. (2014) and Lu et al. (2018) who found that a higher air velocity was helpful for spreading the disinfectants through indoor space as well as to enhance

disinfection efficiency accordingly. This might be because the minimum airflow rate of 0.3 m/min detected in this study was already sufficient enough to thoroughly spread the disinfectant solution in the room space. The results confirmed that ClO₂ disinfection could sufficiently suppress the numbers of bacteria and fungi present in the bioaerosols to comply with the Taiwan EPA guidelines for the indoor air quality.

Bioaerosols on sports equipment

Initial concentration of microorganisms

Table 3 shows the initial concentrations of bacteria and *E. coli* before cleaning the sports equipment. The initial concentrations of bacteria and *E. coli* were different among each experiment because it is based on the number of people who exercised with the specific equipment namely bicycle handle (rubber surface), dumbbell (rough steel surface), and sit-up bench (synthetic leather surface) in the fitness center prior to

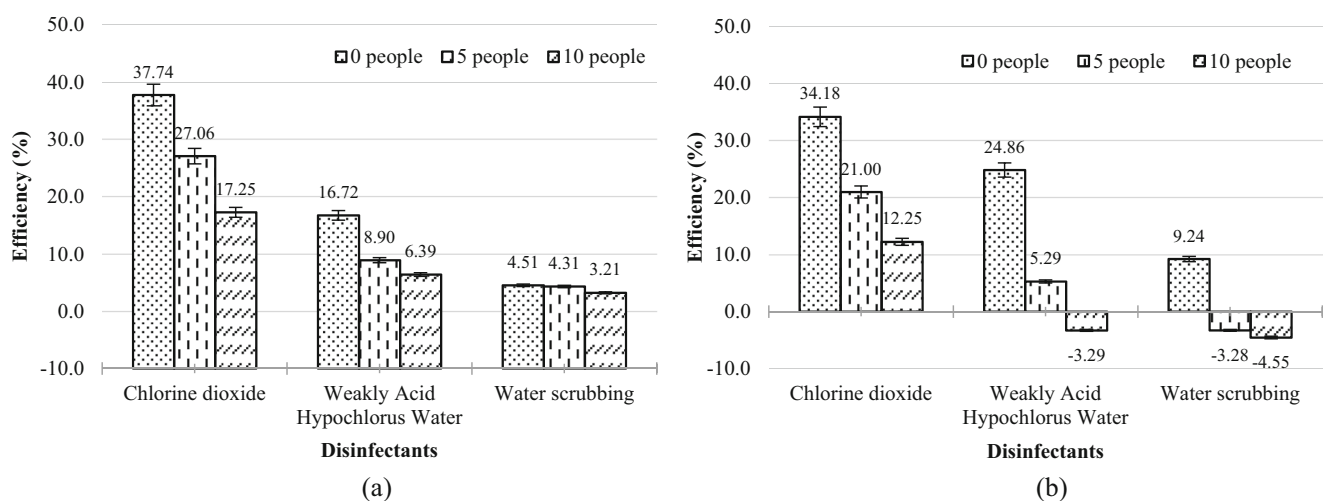


Fig. 3 Effectiveness of fungi disinfection (a) after 15 min and (b) after 60 min (N = 2 or 3)

Table 3 Initial concentration of bacteria and *E. coli* on sports equipment, $N = 2$ (mean \pm SD)

	ClO ₂	WAHW	ZnO	Commercial disinfectant
Initial concentration of bacteria (CFU/cm ²)				
Bicycle handle	756 \pm 278	390 \pm 2	3720 \pm 736	1132 \pm 430
Dumbbell	684 \pm 232	1070 \pm 144	1596 \pm 418	860 \pm 260
Sit-up bench	820 \pm 28	816 \pm 210	840 \pm 566	1486 \pm 234
Initial concentration of <i>E. coli</i> (CFU/cm ²)				
Bicycle handle	990 \pm 146	1080 \pm 114	628 \pm 266	550 \pm 70
Dumbbell	786 \pm 50	700 \pm 28	732 \pm 118	1002 \pm 224
Sit-up bench	866 \pm 94	802 \pm 286	960 \pm 170	910 \pm 14

Remark: “N” = 2

the testing period. It is necessary to note that the experiments were conducted at existing conditions and at different times.

Factors affecting microbial inactivation on sports equipment

Table 4 shows the correlations between the bacteria and *E. coli* disinfection and the related factors. Significant positive correlations were found between the effectiveness of bacteria disinfection and the effectiveness of *E. coli* disinfection ($r = 0.437$, $p < 0.01$) and types of equipment ($r = 0.446$, $p < 0.01$). Significant positive correlations also existed between the effectiveness of *E. coli* disinfection and types of equipment ($r = 0.396$, $p < 0.01$). In other words, the effectiveness of bacteria and *E. coli* disinfection depends on the surface characteristics of sports equipment. Sports equipment selected in this study had different surface characteristics: (1) the handles of bicycle is soft with foam-like surface, (2) the dumbbell is steel and has a rough surface, and (3) the sit-up bench is covered with leather and has smooth surface. Other factors including time and types of the disinfectants had no statistically significant impact on both bacteria and *E. coli* inactivation.

Effectiveness of bacteria and *E. coli* disinfection

Figures 4 and 5 show the efficiency of the four disinfectants in reducing bacteria and *E. coli* present on the sports equipment surface after applying for 2, 5, 10, and 30 min. The results show that all the disinfectants are highly effective in reducing bacteria and *E. coli* present on sports equipment (about 66.8–

95.4%) even within a short period of time after application (2 min).

Figures 4(a) and 5(a) show that chlorine dioxide is highly effective in reducing bacteria and *E. coli* present on sports equipment (81.0–93.9%). It can be seen that ClO₂ could remove bacteria and *E. coli* immediately after 2 min of application. Therefore, users can use this equipment straightaway after disinfection without a long-waiting period. According to Cho et al. (2017), chlorine dioxide (ClO₂) has emerged as an alternative because it has better antimicrobial effectiveness with a higher solubility, shorter response time, and wider pH range. Moreover, the inactivation effectiveness of ClO₂ on both bacteria and *E. coli* was not significantly affected by surface characteristic variation among the sports equipment. Hence, it can be used as a common disinfectant for all equipment in the fitness center. WAHW appeared to be less slightly effective in bacteria and *E. coli* inactivation than ClO₂ as illustrated in Figs. 4(b) and 5(b). Required contact time for WAHW seemed to be 10 min which was much longer than those of ClO₂. This observation is in agreement with the studies of Block and Rowan (2020) and Quan et al. (2017) who stated that hypochlorous acid required at least 10 min of contact time to be effective for antimicrobials.

Zinc oxide is highly effective in reducing bacteria for all of sports equipment as shown in Figs. 4(c) and 5(c). However, it is more effective in reducing *E. coli* on bicycle handle and sit-up bench than dumbbell. Comparing to ClO₂ and WAHW, it was found that ZnO has a slightly better performance on bacteria and *E. coli* disinfection. Nonetheless, direct application

Table 4 Correlations between inactivation effectiveness and environmental factors on sports equipment

	% efficiency on bacteria disinfection	% efficiency on <i>E. coli</i> disinfection	Types of Equipment	Disinfectant
% efficiency on <i>E. coli</i> disinfection	0.437**			
Equipment	0.446**	0.396**		
Disinfectant	0.092	0.009	0.000	
Time	−0.097	0.040	0.000	0.000

**Correlation is significant at the 0.01 level (2-tailed)

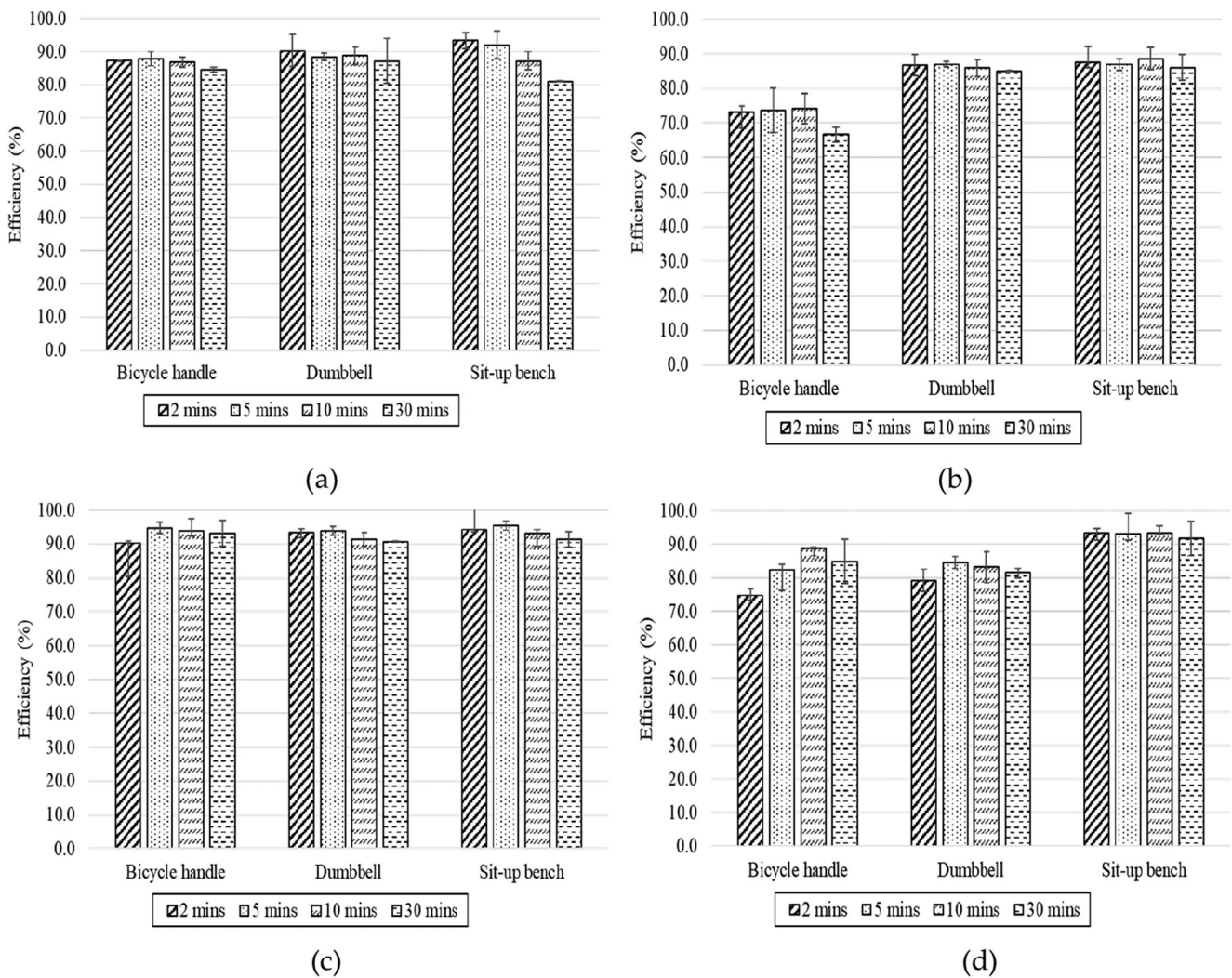


Fig. 4 Effectiveness of bacteria disinfection after cleaning by four disinfectants ($N = 2$). (a) Chlorine dioxide, (b) WAHW, (c) zinc oxide, and (d) commercial disinfectant

of ZnO nanoparticle aqueous solution in indoor environment may cause adverse health impact. ZnO nanoparticles remaining after water evaporation can be suspended by air movement and disperse in the air over a long period of time in the exercise room. Inhalation of these nanoparticles was found to cause lung inflammation and other negative effects in mice (Chen et al. 2015; Larsen et al. 2016).

Commercial disinfectant, which contains cetrimide and chlorhexidine digluconate, could inactivate bacteria and *E. coli* on sit-up bench better than bicycle handle and dumbbell as shown in Figs. 4(d) and 5(d). The commercial disinfectant seems to be suitable for the sports equipment which has smooth surface like sit-up bench rather than rough surface as in the case of bicycle handle and dumbbell.

In summary, all disinfectants could remove bacteria and *E. coli* immediately after application, although the highest disinfection efficiency occurred at different contact times. Nonetheless, it implies that users can use these equipment

straight-away after disinfection without a long waiting period. Chlorine dioxide and ZnO had comparable inactivation effectiveness regardless of sports equipment and surface roughness as shown in Fig. 4(a) and (c), respectively. It is interesting to observe that WAHW and commercial disinfectant could disinfect total bacteria on the sit-up bench slightly better than handles of the bicycle and dumbbell which had rougher surface as shown in the Fig. 4(b) and (d), respectively. The results also demonstrated that the effectiveness of disinfectants somehow depends on the surface characteristics of sports equipment as well.

The *E. coli* disinfection of all disinfectants are quite comparable although the commercial disinfectant showed noticeable less effectiveness on the handles of the bicycle as shown in Fig. 5. Coupled with the findings from bioaerosol disinfection as reported earlier, ClO_2 seems to be a better choice for sanitizing the sports equipment since it was also found to be highly efficient for bioaerosol disinfection as well. Although

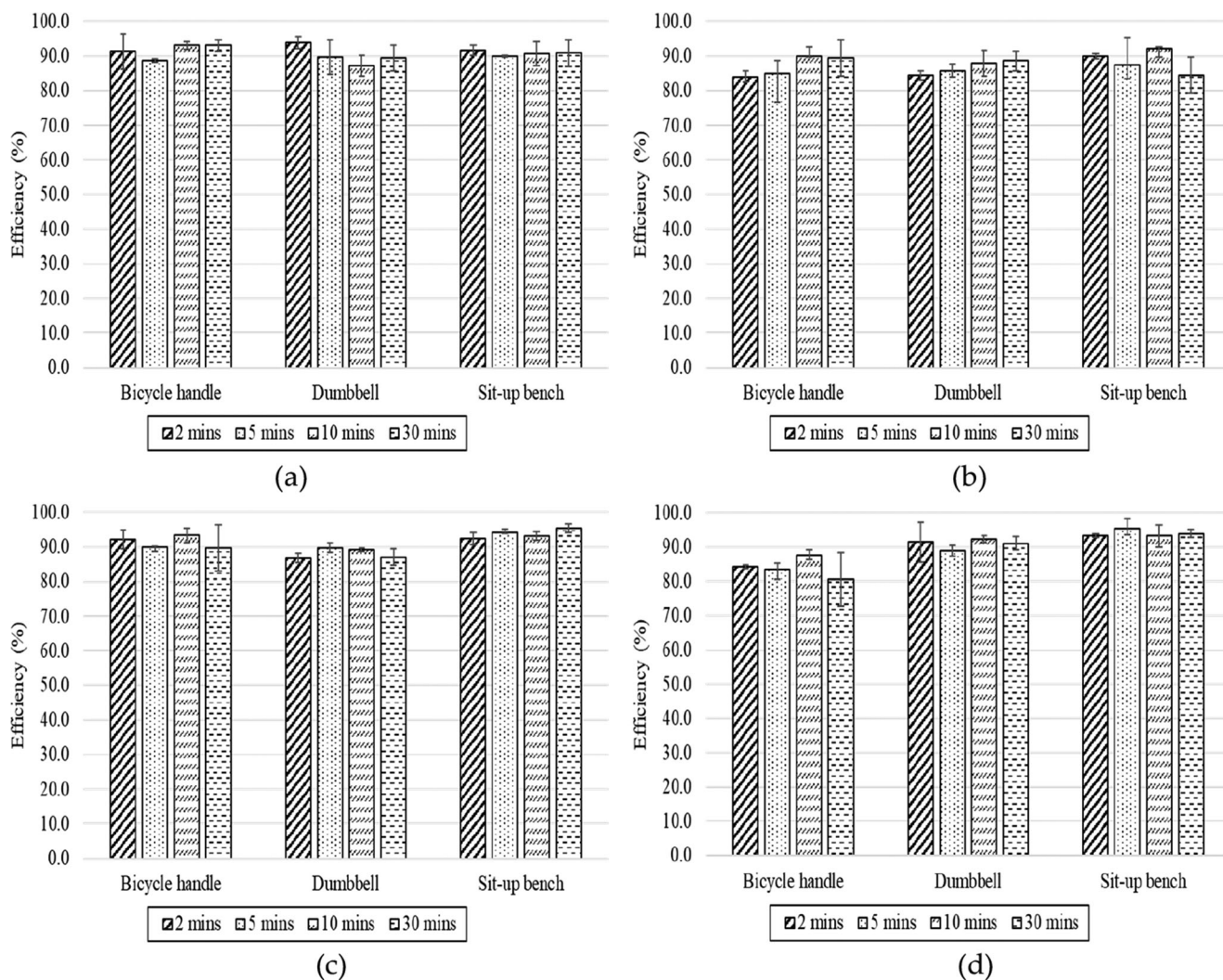


Fig. 5 Effectiveness of *E. coli* disinfection after cleaning by four disinfectants (N = 2). (a) Chlorine dioxide, (b) WAHW, (c) zinc oxide, (d) commercial disinfectant

ZnO and commercial disinfectant were effective for surface disinfection, they are not appropriate nor recommended to apply aerially for bioaerosol disinfection since the former are nano-powders which can cause irritation and toxicity to the respiratory system whereas the latter is primarily produced for surface antiseptic.

It is important to note that this study was carried out during the time of COVID-19 pandemic; thus, the number of users drastically was reduced. According to Kalogerakis et al. (2005), the most important source of airborne bacteria is the presence of people who come to exercise. Particular activities like talking, sneezing, coughing, walking, washing, and toilet flushing can generate airborne biological particulate matter. Therefore, the initial concentrations of bacteria detected in this study were not as high as it should be under normal condition. Nonetheless, the effectiveness of each disinfectant on targeted microorganisms is still valid and can be further applied for normal condition with certain modifications.

Conclusions

This study investigated the effectiveness of bioaerosol disinfection in a fitness room after usage by using ClO_2 , WAHW, and water. Our results indicate that ClO_2 was more effective on fungi inactivation whereas WAHW was better for bacteria disinfection. Water had the least efficiency in removing bioaerosols from the air as expected via physical scrubbing, not disinfection. Number of users statistically diminished the bacteria and fungi removal efficiencies during the experimental period of 60 min for all tested schemes. According to the outcomes, disinfecting the air in the fitness room by ClO_2 aerosolization for 15 min after usage seems to be the most appropriate option for both bacteria and fungi control in bioaerosols in order to improve the air quality in the indoor environment and human health. For microorganisms on sports equipment control, it was found that ClO_2 and ZnO were noticeably better disinfectants than WAHW and commercial

disinfectant in terms of bacteria inactivation whereas all chemicals had comparable effectiveness on *E. coli* disinfection. Targeted microorganisms were effectively inactivated in 2 min after application. Concerning on overall disinfection performance of both bioaerosols and surface of sports equipment, ClO_2 was found to be the most appropriate and effective disinfectant.

Availability of data and materials All data generated, used, and analyzed during this study are included in this article.

Author contribution MCL conceptualized and designed the study, secured the funding of the research, and planned the research activities. He is the corresponding author. NB contributed to the conceptualization and design of the study, supervised the implementation of the research activities, and mentored the other members of the research group. SY and FJL performed the experiments and data collection and initial analysis of the data, and prepared the initial draft of the manuscript. LMB performed some analysis of the data, reviewed, edited, and critically revised the manuscript. All authors read and approved the final manuscript for submission.

Funding This research was made possible through the funding grant by the Ministry of Science and Technology, Taiwan, MOST 107-2622-E-041-002-CC3.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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