Contribution of bioaerosol emission from air-conditioning and mechanical ventilation system to indoor bioaerosol concentration

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**CONTRIBUTION OF BIOAEROSOL
EMISSION FROM AIR-CONDITIONING AND
MECHANICAL VENTILATION SYSTEM TO
INDOOR BIOAEROSOL CONCENTRATION**

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Summary

Human exposure to indoor bioaerosols has been a major health concern indoors. Bioaerosol emission from air-conditioning and mechanical ventilation (ACMV) systems has a potential to be a significant sources of indoor bioaerosols. In order to understand impacts of bioaerosol emission from the ACMV system on indoor occupants, quantification and species identification of bioaerosols emitted from the ACMV system are necessary.

Airborne bacteria and fungi are common bioaerosols indoors. This work investigates bacterial and fungal emissions from an ACMV system in a tropical indoor environment. A series of experiments employing both surface and air sampling are conducted to quantify the emission rates of different bioaerosol sources that contribute to indoor bioaerosol concentrations. Surface sampling is conducted to quantify the loadings of bacteria and fungi on the surfaces of indoor environment and the various components of the ACMV system, and the results indicate that bacterial and fungal accumulation on surfaces of fan blades are the highest among the sampled surfaces. The average loadings of bacteria and fungi on fan blades are more than six and eleven times that on a fresh air duct. Air sampling is performed to measure indoor airborne bacterial and fungal concentrations as well as species. Material balance model is applied to quantify the contribution from ACMV system to indoor bioaerosol concentration. Air sampling results show that both the AC unit and the fresh air duct can emit

bioaerosols into the indoor environment. In addition, the experimental results indicate that emissions from the ACMV system and occupants contribute significantly to indoor airborne bacterial concentrations. Indoor airborne fungal pollution is predominantly contributed by the infiltration of outdoor airborne fungi. Results of simulation of cleaning the ACMV system show that the average indoor airborne bacterial and fungal concentrations can drop by 45% and 34%, respectively, when bioaerosol emission from the ACMV system is removed. This investigation provides direct support that bioaerosol emission from ACMV systems can degrade indoor air quality and that some species could potentially be pathogens for indoor occupants. Natural ventilation is also found to be more effective at lowering indoor airborne bacterial concentrations and less effective at lowering indoor airborne fungal concentrations than ACMV in the indoor space according to air sampling results. Simulation results indicate that the ACMV system can be more effective in lowering both airborne bacterial and fungal concentrations than the natural ventilation system by cleaning the ACMV system employed in the ACMV. The scheme developed in this study to quantify bioaerosol emission from ACMV system, as well as other sources, overcome the limitation of quantification of the bioaerosol emission by resuspension model, which are difficult to be applied on surfaces of non-uniform distribution of microorganisms, for example, most surfaces in air path in real-site ACMV system. The scheme developed in this study to species-identify bioaerosol emission from ACMV system also provide a more comprehensive bioaerosol emission profile compared to the surfaces sampling

method on limited surfaces in the air path, which are widely applied in previous studies. The results of this study will serve as the basis for future indoor bioaerosol exposure estimation and to develop effective control measures against the bioaerosol emission from ACMV systems. In addition, the results of this study also contribute to understanding of applicability of natural ventilation as an alternative ventilation strategy compared to ACMV system, which has a potential to save the significant electricity energy cost by ACMV system. Future work will be conducted to investigate impacts of environmental parameters on comparison of ACMV and natural ventilation. In addition, acoustic agglomeration effects will be investigated as a novel cleaning technology of ACMV system, which has the potential to remove bioaerosol pollution from ACMV system effectively while saving the electricity energy cost.

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List of Symbols

Nomenclature

A	Floor area (m^2)
C	Bioaerosol concentration (CFU/m^3)
C_i	Indoor bioaerosol concentration (CFU/m^3)
$C_{i,1}$	Contribution of initial bioaerosol concentration (CFU/m^3)
$C_{i,2}$	Contribution of bioaerosol resuspension from the floor (CFU/m^3)
$C_{i,3}$	Contribution of the potential bioaerosol emission sources including occupants, the fresh air duct and the AC unit and infiltration of outdoor bioaerosols (CFU/m^3)
CE_i	Experimental results of indoor bioaerosol concentrations
E_0	Infiltration rate of outdoor bioaerosols (CFU/s)
E_1	Bioaerosol emission rate of occupants (CFU/s)
E_2	Bioaerosol emission rate of the AC unit (CFU/s)
E_3	Bioaerosol emission rate of the fresh air duct (CFU/s)
F	Sampling air flow rate of the impactor (14.15 L/min)
N	Bacterial or fungal loadings on surfaces (CFU/m^2)
N_{floor}	Bacterial and fungal loadings on the floor (CFU/m^2)
N	Number of colonies on an agar plate (CFU)
R_{floor}	Resuspension rate of bacteria and fungi from the floor ($\#/s$)

r	Ratio between the volume (100 μL) of liquid sample on an agar plate and the volume (10 mL) of eluted ultra-pure water from one swab (0.01)
S_t	Template area of surface sampling (0.005, m^2)
t	Time (s)
t_0	Sampling time for each sample (10 min)
Q_f	Fresh air flow rate (m^3/s)
Q_r	Return air flow rate (m^3/s)
V	Volume of indoor space (m^3)
V_d	Deposition velocity of indoor bioaerosols (m/s)

Greek Symbols

η_l	Filtration efficiency of the return air filter inside the AC unit
η_f	Fan efficiency
η_m	Stepper motor efficiency

Chapter 1 Introduction

1.1 Motivation

People in developed countries spent almost 90% of their time indoors (Pitarma et al., 2017). Exposure to indoor air pollution cannot only have negative impacts on human productivity but also cause health problems. For example, a study conducted in Denmark and Singapore estimated that the poor indoor air quality could lead to 6-9% drop on office work performance (Wyon, 2004). In India, Health burden in terms of sick days attributable to indoor air pollution was estimated to be 1.6 to 2.0 billion days in a year (Smith, 2000). Exposure to indoor air pollution can increase risk of infants and susceptible adults' specific symptoms like low-birth weight, perinatal mortality, asthma, otitis media, tuberculosis, nasopharyngeal cancer, cataracts, blindness and cardiovascular diseases (Bascom et al., 1994). Non-specific symptoms including eye, nose and throat inflammation, skin irritation and some flu-like symptoms, which are defined as sick building syndrome (SBS) by the World Health Organization (WHO) (Bholah et al., 2000), are also associated with exposure to indoor air pollution .

Among various types of indoor air pollutants, bioaerosol is receiving increasing attention. Exposure to bioaerosols can cause or exacerbate various diseases. For example, previous studies reported that exposure to bioaerosols was the

potential cause of asthma, rhinitis (Beaumont, 1988; Fung and Hughson, 2003) and hypersensitivity pneumonitis (Siersted and Gravesen, 1993). Asthma, a common respiratory disease associated with exposure to bioaerosols, is found in 7.5% of the total population in the US and its prevalence has increased 60% over the past 25 years (Peccia and Hernandez, 2006). Rhinitis, a another common respiratory disease, affects 40 to 60 million people and causes 3.8 million people to lose their jobs every year (Dykewicz and Hamilos, 2010; Elisa, 2007). In addition to being cause of asthma, exposure to indoor bioaerosols was also found to potentially exacerbate asthma symptoms in children and susceptible adults (Park et al., 2001). A previous study found that exposure to airborne mite allergen could aggravate asthma symptoms in up to 85% of asthmatics (Platts-Mills and Carter, 1997). In addition, exposure to indoor airborne fungi has also been reported to be associated with exacerbation of asthma due to its allergic effects in many previous studies (Hintikka and Nikulin, 1998).

Air conditioning and ACMV (ACMV) systems are commonly used to achieve comfortable indoor environment, especially in tropical areas like Singapore, where ACMV systems are widely used for the purpose of cooling and dehumidification (Wu et al., 2016). In addition to thermal comfort, an ACMV system serves as an engineering means to control indoor aerosol level including bioaerosols. In Singapore, the percentage of household, which owned ACMV

systems, has increased significantly in the recent decades from below 10% in 1970-80's to almost 70% in 2000-2010's which is shown in Figure 1.1 (Chua and Chou, 2010; Wong et al., 2002). It is a reasonable hypothesis that this percentage in Singapore can maintain the same level or higher after 2010.

Figure 1.1 ACMV systems installation by households in Singapore (Chua and Chou, 2010)

In a mechanically-ventilated indoor space, an ACMV system can impact indoor bioaerosol dynamics in four aspects: 1) draw outdoor bioaerosols into indoor space through fresh air path by air exchange (Nazaroff, 2014), 2) remove indoor bioaerosols by exhausting indoor bioaerosols to outdoor space through

exhaust air path by air exchange and filtering bioaerosols in the return air using the filter equipped in the ACMV system (Nazaroff, 2014), 3) distribute indoor bioaerosols by controlling flow rate and direction of supply air (Hoge et al., 1994; Li et al., 2007; Menzies et al., 2000; Moser et al., 1979; Riley et al., 1962; Schulman and Kilbourne, 1962; Tang et al., 2006), 4) impact growth or decay of indoor bioaerosols by controlling indoor temperature and RH (relative humidity) (Chen and Hildemann, 2009; Górny et al., 1999; Pasanen et al., 2000).

In addition, it is a reasonable hypothesis, although not yet well established, that bioaerosols can be emitted from an ACMV system to indoor space when it is not well maintained, which provides environments for microorganisms to grow and accumulate on surfaces of the ACMV system (Batterman and Burge, 1995). Many factors, including air movement, mechanical disturbance, movement of components and droplet splash, can lead to the aerosolization of these microorganisms to form bioaerosols (Batterman and Burge, 1995). Then these bioaerosols may be carried into indoor environments by supply air. However, direct evidence of bioaerosol emission from ACMV systems is still lacking. Specifically, increase of indoor bioaerosol concentrations due to bioaerosol emission from the ACMV system has not been observed and bioaerosol emission of the ACMV system is still not quantified and species-identified, which makes it debatable that whether the ACMV system is helping to control

indoor bioaerosol level or is worsening indoor air by emitting bioaerosols to indoor space. In addition to being potential bioaerosol emission source for the indoor space, the operation of ACMV systems accounts for nearly half of the total building electricity consumption in buildings under temperate climate and this can reach 70% for buildings in the tropics, where ACMV systems usually operate for longer time in a day compared to their operation in temperate indoor environment (Ng et al., 2017). Therefore, it is necessary to develop new approaches to avoid the significant energy consumption of operation of the ACMV system while control indoor pollutants level effectively.

Natural ventilation has the potential to be an alternative ventilation approach of the ACMV. Compared to ACMV, natural ventilation can avoid energy consumption due to operation of the ACMV system and potential bioaerosol emission from the ACMV system. However, difference in AER (Air Exchange Rate) between the naturally-ventilated and the mechanically-ventilated indoor space may also impact comparison of their relative effectiveness of controlling indoor bioaerosol concentrations due to the fact that infiltration of outdoor bioaerosols, which is an important source of indoor bioaerosols, is positively related to AER of the indoor space. In addition, compared to ACMV, absence of cooling and dehumidification provided by the ACMV system for indoor space under natural ventilation can also affect their relative effectiveness of controlling indoor bioaerosol concentrations, which are closely associated with indoor temperature and RH. Therefore, whether the natural ventilation is more

effective in lowering indoor bioaerosol concentrations than ACMV is still unknown. In order to tell the applicability of natural ventilation as an alternative ventilation strategy of ACMV, it is of significant importance to investigate the relative effectiveness of controlling indoor bioaerosol concentrations under natural ventilation compared to ACMV.

In order to fill up the above discussed knowledge gaps, the knowledge hierarchy of this work is drawn (as shown in Figure 1.2). The dash-line rectangles (with white background) are the knowledge gaps to be filled by this study, while the dash-line rectangles (with green background) are the knowledge available from existing literature. Quantification and identification of the bioaerosol emission from the ACMV system can contribute to completing understanding of contribution of ACMV systems to indoor bioaerosols and analyzing relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV. The ultimate goal of this research (as shown by the yellow-dash-line rectangle on top) is to explore applicability of natural ventilation as an alternative ventilation strategy of ACMV to lower indoor bioaerosol concentrations while saving energy cost.

Figure 1.2 The knowledge hierarchy of this work.

1.2 Study Objectives and Scopes

1.2.1 Objectives

The ultimate goal of this research is to explore applicability of natural ventilation as an alternative ventilation strategy of ACMV to control indoor pollutant level effectively while saving energy cost. To achieve this goal, potential of bioaerosol emission from ACMV system will be firstly evaluated as a source of indoor bioaerosols. Then the bioaerosol emission from ACMV system will be quantified and species-identified to investigate its health effects,

which can serve as basis of applicability of natural ventilation as an alternative ventilation of ACMV system in controlling indoor bioaerosol level. By comparison of indoor bioaerosol level under natural ventilation mode and ACMV mode, whether natural ventilation can serve as better ventilation strategy of ACMV system can be identified.

1.2.2 Scopes

In order to achieve the objectives, the specific scope of this work is as follows:

- (1) Bioaerosol pollution in the ACMV system will be investigated in a tutorial room to evaluate its potential to be a bioaerosol emission source for indoor space. It will be achieved through several sub-scopes. (Chapter 3)
 - a. Biological particles on surfaces of components in the fresh air and return air path of the ACMV system will be sampled by surface sampling method in the tutorial room.
 - b. Surface samples will be analyzed by cultured-based method to quantify the biological loadings on the sampled surfaces in the ACMV system.
 - c. The biological loadings on the sampled surfaces will be analyzed and compared to evaluate potential of the components as bioaerosol emission sources for indoor space.
- (2) Bioaerosol emission from the ACMV system will be quantified and species-identified to understand its health effects and contribution to the

indoor bioaerosol concentration. It will be achieved through several sub-scopes.

- d. Common species of indoor bioaerosols and their effects on human health will be investigated from the literature. (Chapter 2)
- e. Indoor bioaerosol concentrations will be measured by air sampling method to investigate impacts of bioaerosol emission from the ACMV system on indoor bioaerosol concentrations. (Chapter 4)
- f. Mathematical model derived from mass-balance model to describe indoor bioaerosol dynamics including sources and sinks of indoor bioaerosols will be developed. (Chapter 4)
- g. Mathematical solution of indoor bioaerosol concentration according to the developed mathematical model (f) will be used to fit experimental data of indoor bioaerosol concentrations to calculate bioaerosol emission rate of the ACMV system. (Chapter 5)
- h. Simulation of variation of indoor bioaerosol concentrations in cases without bioaerosol emission from the ACMV system will be applied to evaluate mitigation of indoor bioaerosol pollution by removing bioaerosol emission from the ACMV system.(Chapter 6)
- i. Air sampling experiments will be conducted to sample bioaerosols originated from ventilation ducts and the AC unit in the ACMV system, and the bioaerosol samples will be analyzed to specify species that originated the from ACMV system by DNA-based technology. (Chapter 7)

- (3) Applicability of natural ventilation as an alternative ventilation strategy of ACMV regarding to indoor bioaerosol control will be investigated. It will be achieved through several sub-scopes as well.
- j. Energy consumption of operation of ACMV systems in the building is investigated from the literature. (Chapter 2)
 - k. Effectiveness of controlling indoor pollutants (except bioaerosols) between natural ventilation and ACMV will be compared from the literature. (Chapter 2)
 - l. Indoor bioaerosol concentrations under natural ventilation and ACMV will be measured by air sampling method to compare their relative effectiveness of lowering indoor bioaerosol concentrations. (Chapter 4)

Chapter 2 Literature Review

Health effects of human exposure to indoor bioaerosols can be decided by characteristics (chemical, physical and biological characteristics) and the amount of indoor bioaerosols, which occupants are exposed to (Douwes et al., 2003b). Characteristics of bioaerosols depend on species of the bioaerosols. In an indoor space, amount of indoor bioaerosols, which occupants are exposed to, is positively related to the indoor bioaerosol concentrations, which are decided by indoor bioaerosol dynamics. Investigation of species of indoor bioaerosols and indoor bioaerosol dynamics is necessary to understand health effects of human exposure to indoor bioaerosols and develop effective approach to control indoor bioaerosol level.

2.1 Indoor Bioaerosols and Their Health Effects

Bioaerosols refers to suspended airborne particles that contain living organism or were released by those organism in indoor environments. Sizes of biological particles range from less than 0.1 μm to 100 μm in aerodynamic diameter (Sánchez-Monedero et al., 2008). Diseases related to exposure to bioaerosols are mainly infectious diseases and respiratory diseases (Douwes et al., 2003a). Infectious diseases are usually caused by transmission of viruses, bacteria, fungi and other biological agents from a reservoir to a susceptible and new host. For example, infection of the occupants in offices, military and other workplaces can be the result of exposure to bioaerosols. Respiratory diseases

are commonly attributable to exposure to airborne dusts and bioaerosols such as toxins, fungal spores and allergens. For example, in a study of the outbreak of SARS in Hong Kong, It was pointed out that the SARS virus was likely to spread through airborne routes (Yu et al., 2004). Some of symptoms of the respiratory diseases are mild and can hardly influence the daily life whereas some are serious ones that need special care. In addition, non-infectious bioaerosol such as pollen can also spread through airborne routes (Cabezudo et al., 1997; Ricci et al., 2005). Ratio of infections caused by airborne spread of pathogen is estimated to be 10 to 20 percent of total endemic nosocomial infections (Brachman, 1970). These bioaerosols and their health effects are summarized in Table 2.1.

Due to adverse health effects are closely associated with concentrations of indoor bioaerosols, the limit concentrations of indoor bioaerosol are recommended by different agencies and organizations in the world as the following: 1000 CFUs/m³ (National Institute of Occupational Safety and Health (NIOSH))(Crook and Burton, 2010), 1000 CFUs/m³ with the total amount of viable bacteria not exceeding than 500 CFUs/m³ (American Conference of Governmental Industrial Hygienists (ACGIH))(Kalogerakis et al., 2005).

Table 2.1 Health effects of bioaerosols

Bioaerosol	Health effects	Statistics
Fungi	Allergy, skin irritation , asthma, rhinitis(Husman, 1996); Headache, joint fever (Rylander and Etzel, 1999); headache and dizziness (Platt et al., 1989); bronchus, paranasal sinus, vertebrate infections (Castelli et al., 1990; Hantsch and Tanus, 1991; Schwartz et al., 1992)	(1)eye irritation, respiratory symptoms associated with molds in 15000 residents in Canada((Dales et al., 1991); (2) The estimated prevalence ratio of mold allergy from 5% to 50% for different populations (Mygind, 1986)
Bacteria	Legionnaires' diseases (McDade et al., 1977); Tuberculosis and leprosy (Burrell, 1991); anthrax (Ivens et al., 1999)	Estimated 8.8 million incident cases and 1.45 million deaths globally in 2010 (Zumla et al., 2012)
Virus	SARS (Yu et al., 2004); Influenza (WHO, 2009)	(1)1175 in the population of 6.7 million were infected and 299 died of SARS with a fatality rate of 17 percent (Yu et al., 2004); (2) Popularity of influenza happens around the world every year and lead to three to five million serious cases and about 250,000 to 500,000 death (WHO, 2009)
Pollen	Pollinosis (Bousquet and Burney, 1993; Wüthrich, 1989); sneezing, runny or	(1) In the US, the prevalent allergic response is hay fever

blocked nose, itchy or watery eyes, an itchy throat, mouth, nose and ears (Ehrenstein et al., 2000) which affects 10% to 20% of the population (Anderson et al., 2004); (2) self-reported cases of hay fever increased more than 10-fold, reaching 14.2% of the population between 1991 and 1993 (D'amato et al., 1998); (3) The prevalence of pollinosis estimated according to a questionnaire-based survey and self-reported data ranges from 4.9% in Spain to 16.5% in the UK (D'amato et al., 1998).

House dust mites	Allergy (American College of Allergy, 2014)	56% of 628 allergic rhinitis patients were sensitive to the house dust mites according to the cross-sectional study in Mexico (Larenas-Linnemann et al., 2014)
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Among all kinds of bioaerosols, airborne bacterial and fungal pollution can cause serious health problems such as airway inflammation (Degobbi et al., 2011) and respiratory symptoms (Hargreaves et al., 2003) and receive considerable concern. They are listed as predominant microbiological agents indoors according to Singapore Standard 554 (2016b). Therefore, airborne bacterial and fungal pollution are investigated as the predominant indoor bioaerosols in this study.

2.2 Indoor Bioaerosol Dynamics under Air-Conditioning and Mechanical Ventilation System

2.2.1 Adding of Indoor Bioaerosols

2.2.1.1 Infiltration of Outdoor Bioaerosols

Infiltration of outdoor bioaerosols can add indoor bioaerosol concentrations. By air exchange, outdoor bioaerosols is drawn into indoor space through fresh air path of the ACMV system. The fresh air flow rate is decided by AER and volume of the indoor space. In the fresh air path, a fresh air filter is commonly used to filtrate bioaerosols carried by fresh air, whose filtration efficiency is decided by quality of the filter and sizes of the bioaerosols (Farr, 2001; Nazaroff, 2014). In addition, deposition of bioaerosols in the fresh air on internal surfaces in the fresh air path in the ACMV system can also lead to loss of infiltration of outdoor bioaerosols, which is decided by deposition velocity of the bioaerosols and area of the internal surfaces in the fresh air path (You and Wan, 2015). Therefore, contribution of infiltration of outdoor bioaerosols to indoor bioaerosol concentrations depends on concentration of outdoor bioaerosols drawn into the fresh air path, fresh air flow rate and loss of bioaerosols due to filtration and deposition in the fresh air path of the ACMV system.

2.2.1.2 Bioaerosol Emission from the ACMV System

According to a questionnaire-based investigation in UK, there were more complaints in buildings equipped with ACMV systems than those without ACMV systems (Burge et al., 1987). In the US, more than half of residents in 484 buildings were not satisfied with controlling effects of indoor air quality by ACMV in the buildings according to a nationwide report (Scitz, 1990). In Canada, ACMV systems were associated with same problems that air quality in mechanically-ventilated indoor spaces are not satisfying (Kirkbride et al., 1990). Based on significant amount of investigations, reporting the association of degradation of indoor air quality and ACMV systems, they were considered to contribute to indoor air degradation (Fanger et al., 1988; Mølhave and Thorsen, 1991; Pejtersen et al., 1991).

Early in 1995, investigation of environment inside ACMV systems hypothesized that RH and temperature inside can be favorable to persistence and growth of microorganisms, where high concentration of odor was found, indicating that the ACMV systems have the potential to be bioaerosol emission sources (Batterman and Burge, 1995). Biological loadings on the surfaces in the air path of the ACMV system can reflect its potential as an source of bioaerosol emission, which is due to bioaerosol resuspension from the surfaces in the air path of the ACMV system, induced by the air flow (You and Wan, 2015). Biological loadings on the surfaces in the air path of the ACMV system come

from bioaerosols attached to the surfaces of the ACMV system when air flows pass through (Seppänen and Fisk, 2004), which can accumulate in the ACMV system when the ACMV system is not well designed, maintained or operated (Balasubramanian et al., 2012; Maus et al., 2001; Seppänen and Fisk, 2004). Both modelling studies (Grigonyte et al., 2014; Siegel, 2002; Tian and Ahmadi, 2007) and experimental study (Waring and Siegel, 2008) revealed that airborne particles can deposit on external surfaces of cooling coils and internal surfaces of ventilation ducts of the ACMV system, when the particles are passing through the air path in the ACMV system carried by air flow, suggesting potential of accumulation of bioaerosols in the ACMV system. Evidences that some microorganisms can survive and grow on surfaces in the air path of ACMV systems (Clausen, 2004; Lu et al., 2009) also support the idea that the microorganisms can accumulate in the ACMV systems. Measurement of biological loadings on surfaces in the air path of an ACMV system can provide intuitive information of the extent that the ACMV system is polluted by microorganisms and potential of the ACMV system as a bioaerosol emission source, and has been adopted in areas such as China (Li et al., 2012) and Europe (Kolari et al., 2005) , Canada (Auger, 1994), where ACMV systems were found to be polluted by microorganisms.

Over the years, quantification of aerosol emission from ACMV systems are carried out by performing experiments (Barth et al., 2014; Kim et al., 2016;

Zhang et al., 2013) and developing mathematical model of aerosol resuspension from surfaces in the air path of ACMV system such as internal surfaces of ventilation ducts and external surfaces of cooling coils (Chatoutsidou et al., 2017; You and Wan, 2015; Zhang et al., 2013). For example, Kim conducted wind tunnel experiments to simulate aerosol resuspension from flat duct surfaces and revealed existence of threshold RH value, below which resuspension rate of particles from the flat surfaces are almost constant (Kim et al., 2016). Zhang developed a resuspension model to describe micro-fine particles resuspension in fully developed turbulent layers (Zhang et al., 2013). You and Wan established a risk assessment scheme by modeling air-induced particle resuspension to simulate bioaerosol emission from the ACMV system to indoor space and quantify impact of air-induced aerosol resuspension from ventilation ducts on indoor particle concentrations (You and Wan, 2015).

Health effects of human exposure to indoor bioaerosols are closely related to species of the bioaerosols. Therefore, identification of bioaerosol species originated from ACMV system is of significant importance to understand health effects of bioaerosol emission from the ACMV system on indoor occupants. Previous investigation of species identification of bioaerosols originated from ACMV systems has been conducted by performing site surface-sampling experiments of biological loadings on surfaces in the air path of the ACMV systems and biological analysis of the surface samples in areas

like China (Lu et al., 2009), Europe (Hussein et al., 2013) and the USA (Foarde et al., 1997) to explore ecological distribution of bioaerosols from the ACMV system in these areas.

Most of the previous studies focused on measurement of the accumulation of microorganisms on ventilation ducts and cooling coils to investigate potential bioaerosol emission from the ACMV system (Schmidt et al., 2012; Siegel and Carey, 2001; Zhao and Wu, 2006) in temperate climate regions and few studies explored the microorganism contamination surfaces of the ACMV system and their emissions to indoor spaces under tropical climate. Current understanding of bioaerosol emission from ACMV systems in a tropical indoor environment, where environmental conditions such as high temperature and RH are favorable to growth of microorganisms (Nazaroff, 2014), is still limited. Specifically, knowledge of biological loadings on surfaces in the air path in ACMV systems is still lacking, bioaerosol emission rate from ACMC systems is still not quantified and knowledge of species profile of bioaerosols originated from ACMV system is still lacking in a tropical indoor environment.

2.2.1.3 Other Sources of Indoor Bioaerosols

For general indoor bioaerosols, indoor emissions sources include bioaerosol emission from human beings, resuspension from microorganism-contaminated

internal surfaces of the indoor space (floor, wall and ceiling), penetration of outdoor bioaerosols through leakage of indoor space, release of bioaerosols from rotted food and damaged furniture, etc. Among these indoor emission sources, bioaerosol emission from human occupants and bioaerosol resuspension from the microorganism contaminated internal surfaces of indoor space caused by human activities are predominant (Nazaroff, 2014). Bioaerosol can be released from humans' skin and clothing (Duguid and Wallace, 1948) and their emission can be contributed by friction between the skin and the clothing of occupants indoors (Hall et al., 1986). Respiratory activities of indoor occupants such as talking and coughing (Hospodsky et al., 2012) can also cause significant bioaerosol emission from occupants (Duguid, 1946; Fennelly et al., 2004; Qian et al., 2012; Stelzer - Braid et al., 2009). However, inhalation of occupants can also be a sink of indoor bioaerosols (Nazaroff, 2014). Therefore, bioaerosol emission from human occupants can be affected by many factors such as environmental temperature, RH and occupants' health condition, and varies in a wide range (Nazaroff, 2014). For example, a previous study (Hospodsky et al., 2015) showed that the emission rates of total (including both culturable and non-culturable) biological particles of a sitting person are $(14 \pm 14) \times 10^6$ /hr (arithmetic mean $\pm$ std.dev) for bacteria and $(14 \pm 21) \times 10^6$ /hr for fungi.

Bioaerosol can attach to particulate matters adhered to surfaces (Alghamdi et al., 2014), which can be detached from air and depose or even grown on internal surfaces in the indoor environment. Daily motional activities of indoor occupants like walking, cleaning and cooking can make those particles suspended (Kalliokoski et al., 1996). Many previous experiments demonstrated a positive relationship between extent of indoor daily activities and bioaerosol concentrations (Chen and Hildemann, 2009). Among the daily activities, walking is reported to be the main cause of resuspension of indoor bioaerosols (Goebes et al., 2011; Tian et al., 2014; You and Wan, 2015). In an indoor environment, resuspension rate of indoor bioaerosols of different sizes can be estimated by data of experiments conducted in previous studies (Thatcher and Layton, 1995; Zhou et al., 2011).

2.2.2 Removal of Indoor Bioaerosols

Indoor bioaerosols can be removed in three ways: (1) by air exchange, indoor bioaerosols can be exhausted through exhausted air path of the ACMV system. Exhausted air flow rate equals to the fresh air flow rate of the indoor space. (2) They can be filtrated by filters installed in the return air path of the ACMV system when the return air passes through carrying the indoor bioaerosols. Efficiency of the return air filter varies with quality of the filter and size of the particles passing through (Farr, 2001; Nazaroff, 2014). (3) Indoor bioaerosols can deposit on internal surfaces of the indoor space (floor, wall and ceiling) and

deposition rate of the bioaerosols can be estimated by an empirical model (Lai and Nazaroff, 2000) or experimental data according to sizes of the indoor bioaerosols (Hussein et al., 2013; Lai, 2002; Zhou et al., 2011).

2.2.3 Impacts of Air Exchange Rate on Indoor Bioaerosol Concentrations

Average indoor bioaerosol concentrations are significantly associated with ventilation rate (Nazaroff, 2014). Many previous studies found significant relationship between AER, health symptoms and airborne transmission of pathogen. For example, inadequate AER was found to be significantly associated with incidence increase of respiratory diseases (Daisey et al., 2003). The Wells Riley equation also indicates that infectious possibilities of indoor pathogen is reversely related to AER (Riley et al., 1978). Low AER was one of important factors that lead to outbreak and spread of diseases in indoor environments (Hoge et al., 1994; Moser et al., 1979).

2.2.4 Impacts of Air Flow Pattern on Transportation of Indoor Bioaerosols

In a mechanically-ventilated indoor space, flow rate and direction of supply air provided by the ACMV system can decide indoor air flow pattern, which is associated with distribution of indoor bioaerosols (Edlin et al., 1992; Kumari et

al., 1998; Leclair et al., 1980; Li et al., 2005a; Meselson et al., 1994; Olsen et al., 2003; Remington et al., 1985; Yu et al., 2004). For example, experiments conducted in offices and hospitals found that spread of pathogen is associated with indoor air flow patterns (Bloch et al., 1985; Gustafson et al., 1982; Hutton et al., 1990; Ignatius et al., 2005; Li et al., 2005b; Wehrle et al., 1970; Wong et al., 2004). Earlier investigation (Langmuir, 1980) on outbreak of smallpox demonstrated that transportation of bioaerosols was related to indoor air movements. In addition, outbreak of some diseases like tuberculosis (Hutton et al., 1990), measles (Bloch et al., 1985) and nosocomial varicellar (Gustafson et al., 1982) in healthcare settings occurred in rooms with positive pressure, which contributes to transmission of bioaerosols from the patients' rooms to others' rooms, indicating significant effects of airflow pattern on transportation of pathogen .

2.3 Energy Consumption of Operation of the Air-Conditioning and Mechanical Ventilation System

The operation of ACMV systems accounts for nearly half of the total building electricity consumption in buildings under temperate climate (Pérez-Lombard et al., 2008) and this can reach 70% for buildings in the tropics, where ACMV systems usually operate for longer time in a day compared to their operation in temperate indoor environment (Ng et al., 2017). In buildings, energy consumption of operation of the ACMV system is predominant compared with

other energy use such as lighting and appliance. For instance, according to data from the American Society of Heating, Refrigerating and Air-Conditioning engineers (ASHRA), the ACMV systems contribute to more than two-thirds of the total energy consumption in hospitals in the USA (Grosskopf and Mousavi, 2014). For the residential buildings (Table 2.2), it consumes more than twice the energy use of domestic hot water.

Table 2.2 Energy consumption by end uses in the residential sector

End uses in the residential sector (%)	Spain	UE	USA	UK
Space conditioning	42	68	53	62
Domestic hot water	26	14	17	22
Lighting and appliances	32	18	30	16
Year 2003. Source: Energy Information Administration(EIA), IDEEA(de Iluminación, 2005) and Building Research Establishment (BRE)				

2.4 Applicability of Natural Ventilation as Alternative Ventilation Strategy of Air-Conditioning and Mechanical Ventilation System

2.4.1 Introduction to Natural Ventilation

Natural ventilation is a ventilation strategy that can avoid energy consumption due to operation of the ACMV system. In addition, an indoor space under natural ventilation usually have higher AER compared to that under ACMV, which can lead to better refreshment of indoor air (Escombe et al., 2007). Compared to ACMV system, which utilizes fans in the ACMV system to achieve air exchange, the air refresh in a naturally-ventilated indoor space is achieved via the windows, louvers in indoor space and largely depends on differences of pressure, which are results of wind movement and buoyancy forces. Both these two Mechanisms rely critically on size and distribution of openings in the envelope. Winds movement leads to the positive pressure on upwind side and negative pressure on the downwind side. Air will move in windward openings and out through leeward openings to make a balance of pressure. The buoyancy forces are caused by differences of temperature. Warmer air whose density is lower is more buoyant and rises above cooler air with greater density.

2.4.2 Comparison of Natural Ventilation and Air-Conditioning and Mechanical Ventilation System in Controlling Indoor Pollutants

Although natural ventilation can save more energy than ACMV, further exploration of their relative effectiveness of controlling indoor pollutants level is still needed to tell applicability of natural ventilation as an alternative ventilation strategy of ACMV. A previous investigation was carried out by performing experiments to compare their relative effectiveness of controlling indoor carbon dioxide, total volatile organic compounds (TVOC) and PM 2.5 level in an office between the condition under natural ventilation and that under ACMV (Montgomery et al., 2015). The experimental results suggested that a combination of natural ventilation and ACMV could be a better ventilation strategy than any of them in meeting indoor air quality (IAQ) needs. A similar study which compared IAQ in naturally-ventilated residential buildings and mechanically-ventilated residential buildings was performed in Singapore and found that sick building syndrome occurs more often in mechanically-ventilated indoor spaces (Montgomery et al., 2015). However, bioaerosols, as important indoor pollutants closely related to occupants' health, is not investigated in these comparisons between natural ventilation and ACMV.

Natural ventilation can avoid potential bioaerosol emission from the ACMV system mentioned in Section 2.2.1.2. However, compared to ACMV, there are

no filters to remove indoor bioaerosols under natural ventilation. Absence of cooling and dehumidification provided by ACMV system for indoor space under natural ventilation may impact its relative effectiveness of controlling indoor bioaerosol concentrations compared to ACMV due to the fact that growth and decay of indoor bioaerosols are significantly associated with indoor temperature and RH (Nazaroff, 2014). In addition, difference in AER between the naturally-ventilated and the mechanically-ventilated indoor space may also impact comparison of their relative effectiveness of controlling indoor bioaerosol concentrations due to the fact that infiltration of outdoor bioaerosols, which is an important source of indoor bioaerosols, is positively related to AER of the indoor space. Therefore, the assessment of priority of natural ventilation system in controlling indoor bioaerosol concentrations compared to ACMV is not consistent in previous studies. For example, early in 1986, bioaerosols in naturally-ventilated homes and mechanically-ventilation homes were sampled and the ecological analysis were conducted in the USA (Kodama and McGee, 1986), where it was found that indoor fungal concentration was higher under natural ventilation system than that under ACMV system. In some other studies, higher ventilation rate found in natural ventilation systems, compared to ACMV system, proved to be more effective to control indoor bioaerosol level (Escombe et al., 2007; Gilkeson et al., 2013; Qian et al., 2010), where natural ventilation systems are applied in some hospitals to reduce infection risk while saving energy cost. However, buildings with well-maintained ACMV system were demonstrated to achieve better control effects of indoor bioaerosols

compared to natural ventilation due to the filtration effects in ACMV system to remove indoor bioaerosols (Parat et al., 1997).

Besides the factors mentioned above, which can affect relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV, climate is another important affecting factor of this comparison due to its close relationship with infiltration of outdoor bioaerosols, which can be affected by ambient conditions such as temperature, RH and rainfall. Most comparisons of relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV up to date including the above studies are conducted in temperate climate. However, few investigations of this comparison are conducted under tropical climate, which make it difficult to evaluate relative effectiveness of lowering indoor bioaerosol concentrations between natural ventilation and ACMV in a tropical indoor environment. Therefore, it is necessary to compare relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV in areas under tropical climate, for example, Singapore, to investigate applicability of natural ventilation as an alternative ventilation strategy compared to ACMV in these areas.

2.5 Conclusion

In a mechanically-ventilated indoor space, an ACMV system can impact indoor bioaerosol dynamics in four aspects: 1) draw outdoor bioaerosols into indoor space through fresh air path by air exchange (Nazaroff, 2014), 2) remove indoor bioaerosols by exhausting indoor bioaerosols to outdoor space through exhaust air path by air exchange and filtering bioaerosols in the return air using the filter equipped in the ACMV system (Nazaroff, 2014), 3) distribute indoor bioaerosols by controlling flow rate and direction of supply air (Hoge et al., 1994; Li et al., 2007; Menzies et al., 2000; Moser et al., 1979; Riley et al., 1962; Schulman and Kilbourne, 1962; Tang et al., 2006), 4) impact growth or decay of indoor bioaerosols by controlling indoor temperature and RH (relative humidity) (Chen and Hildemann, 2009; Górný et al., 1999; Pasanen et al., 2000). In addition, an ACMV system has the potential to be a bioaerosol emission source for indoor space.

In order to investigate bioaerosol emission from ACMV systems, most of the previous studies focused on measurement and analysis of biological loadings on internal surfaces of ventilation ducts and external surfaces of cooling coils (Schmidt et al., 2012; Siegel and Carey, 2001; Zhao and Wu, 2006) in temperate climate regions. Few studies explored the microorganism contamination surfaces of the ACMV system and their emissions to indoor spaces in areas under tropical climate, which make current understanding of

potential of bioaerosol emission from ACMV system and its contribution to indoor bioaerosol concentration limited in these areas. Specifically, quantification and species-identification of bioaerosols emitted from the ACMV system need to be conducted in tropical indoor environment. In addition, comparison of relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV is still not investigated in a tropical indoor environment, which makes it difficult to evaluate applicability of natural ventilation as an alternative ventilation strategy of ACMV.

Chapter 3 Experimental Study of Bioaerosol Emission from Air-Conditioning and Mechanical Ventilation System

3.1 Investigation of Biological Loadings on Surfaces in the Air Path of the Air-Conditioning and Mechanical Ventilation System

3.1.1 Introduction

Over the years, measurement of biological loadings on surfaces in the air path of the ACMV system was conducted in temperate areas such as China (Li et al., 2012), Europe (Kolari et al., 2005) and Canada (Auger, 1994). However, knowledge of biological loadings in ACMV systems is still lacking in areas under tropical climate, where environmental conditions such as high temperature and RH are favorable to growth of microorganisms (Nazaroff, 2014). In this chapter, measurement and analysis of biological loadings on internal surfaces of the fresh air duct, a return air filter, and fan blades in an ACMV system is performed in a tutorial room in Singapore to evaluate potential of the ACMV system as a bioaerosol emission source for indoor space.

3.1.2 Experimental Setup and Investigation

3.1.2.1 Experimental Site

Experiments were conducted in a tutorial room (6.30 m (L) x 5.80 m (W) x 2.80 m (H)) located at Nanyang Technological University (NTU), Singapore. A schematic diagram of the tutorial room is shown in Figure 3.1. The ACMV system of the room consists of a ceiling cassette type direct expansion air-conditioning (AC) unit (plfy-p63vam-e, MITSUBISHI ELECTRIC) and a fresh air duct. The AC unit draws room return air through a grill that has a Minimum Efficiency Reporting Value (MERV)-4 rating return air filter installed. The AC unit also draws outdoor air via a 22.8 m-long fresh air duct. The duct consists of a 20.8-m long galvanized steel section and a 2-m long insulated flexible duct section. There is a fresh air fan in the fresh air duct at around 4 m downstream of the fresh air intake. There is a fresh air filter of MERV-8 rating installed at about 3.2 m downstream of the fresh air intake. The supply air flow rate of the AC unit is 1400 m³/hr, constant air volume. The ACMV system was installed about two years before the experiments. The ACMV system generally works 7 - 8 hours per day, 5 days per week. Indoor air temperature set point is 24°C and relative humidity (RH) set point is 60% when the AC unit is switched on. The last major cleaning/servicing of the fresh air duct took place around a year before the experiments. The AC unit, the return air filter and the fresh air filter have never been cleaned/serviced since they installed. The tutorial room can also be operated in natural ventilation mode. The ACMV system generally

works seven hours per day, five days per week under its working condition. Before the start of the whole experiment, the tutorial room was used for teaching from 9:30 am to 4:30 pm with occupancy from four to eight students every day, and cleaned once a day. During the experimental period, the tutorial room was only used for the experiment and was cleaned by the researcher after every day's experiment. There is a sliding window (2.5 m^2) on the wall. When the room is naturally-ventilated, the window is open and the AC unit is switched OFF.

Figure 3.1 Schematic diagram of the room in the experiments

3.1.2.2 Agar Preparation and Transportation

A culture-based method is employed for the biological analysis of this study. Tryptic soy agar (SIGMA-ALDRICH) and malt extract agar (SIGMA-ALDRICH) are used for bacterial and fungal cultivation, respectively. In preparation of agar plates for subsequent biological (bacterial and fungal) surface and air sampling, agar powder (50 g of malt extract agar or 40 g of tryptic soy agar) is suspended in one litre of ultra-pure water produced by a water purification system (Direct Q@5 UV, EMD Millipore). The agar suspension is put into an autoclave (HG-80, HIRAYAMA) for 2-h sterilization and mixing. The agar suspension is then poured onto petri dishes in a class II biosafety cabinet (Nu-440-400E, NUAIR). The petri dishes are left in the biosafety cabinet for 30 minutes under UV light (to avoid biological contamination) to allow agar solidification and form agar plates. The agar plates are then sealed by a film (PM 996, PARAFILM) and stored in a refrigerator (GR-262SVQ, LG) at a temperature of 4°C. Agar plates for surface sample culturing are prepared by the same method, except that these plates do not need to be transported to the sampling site. After sampling, the sampled agar plates are sent to incubator for incubation of the sampled microorganisms. Bacterial samples (Tryptic soy agar as petri dish) are incubated under temperature of 35 °C for 48 hours and fungal samples (Malt extra agar as petri dish) are incubated under temperature of 27 °C for 72 hours.

3.1.2.3 Surface Sampler

SKC sterile surface swap kit (Surface Swap Kit, SKC) is used for the measurement of biological loadings on surfaces in the air path of the ACMV system. It contains a plastic template with 5 cm × 10 cm to define the area of surface sampling, and a sterile foam swab stored in a sterile storage tube, as shown in Figure 3.2. Sampling by the SKC sterile surface swap kit is non-destructive for the microorganisms on the sampled surfaces and can be used safely on most surfaces including irregular surfaces, which are commonly found on components in the ACMV system. Therefore, the SKC sterile surface swap kit is ideal for measuring surface biological loadings.

Figure 3.2 Surface sampler comprising plastic template and sterile foam swab.

3.1.2.4 Biological Surface Sampling

In order to characterize the bacterial and fungal contamination levels on the surfaces of various components of the ACMV system, surface samplings using swab kits (Surface Swap Kit, SKC) are conducted. Surfaces of the return air filter in the AC unit, the fan blades in the AC unit, the internal surfaces of the insulated flexible duct, and the galvanized steel duct are sampled, (as shown in Figure 3.3). For the return air filter and the fan blades in the AC unit, the surfaces facing the incoming return airflow are sampled. For each type of

surfaces, three targeted area are randomly selected for surface sampling to investigate bioaerosol loadings on each type of surfaces. In addition, surface sampling is also performed on the floor of the tutorial room because bioaerosols deposited on the floor can also become airborne and a source of indoor bioaerosols due to resuspension (Zhou et al., 2011).

ACMV system

Figure 3.3 Surface sampling points in the ACMV system

A swab kit includes a sterile foam swab and a 5 cm × 10 cm plastic template that defines the targeted area. For each surface, three randomly-selected areas

are sampled using three different swab kits. During a surface sampling, the target area defined by the template is swabbed thoroughly in a rolling mode. The sampling process of the surfaces is illustrated in Figure 3.5. After sampling, the foam swab is kept in the sterile storage tube that comes with the kit and is then transported back to the laboratory for culture-based analysis. The procedure of the culture-based analysis is illustrated in Figure 3.5. To extract the surface sample from the swap kit, 10 mL of ultra-pure water is added to each storage tube containing the sampled swabs. The tubes are then vortex-shaken in a vortex mixer (MaxiMixII, SPD Scientific) for 1 min. The liquid sample eluted from the swab is then transferred to a clean tube. Next, 100 μ L of the liquid sample in the clean tube is extracted by a pipette (Labopette, HIRSCHMANN) and distributed on an agar plate (tryptic soy agar for bacteria and malt extract agar for fungi) for cultivation. Five repeats of agar plates are made for each eluted liquid sample. The bacterial plates are incubated (Incucell 111, MMM) at 35°C for 48 hours whereas the fungal plates are incubated in another incubator (FOC 215I, VELP Scientifica) at 27°C for 72 hours (2016b). After the incubation, the number of colonies on each agar plate is visually counted on a colony counter (SC 6, STUART). Bacterial/fungal concentration on the sampled surface (N , colony forming unit (CFU) per square meter) can be calculated as $N = n / (r \cdot S_t)$, where n (CFU) is the number of colonies on an agar plate. $r = 0.01$ is the ratio between the volume (100 μ L) of liquid sample on an agar plate and the volume (10 mL) of eluted ultrapure water from one swab. $S_t = 0.005, \text{m}^2$ is the area of the plastic surface sampling template.

Figure 3.4 Sampling process of bioaerosols deposited on surfaces.

Figure 3.5 Sampling and analysis processes of bioaerosols deposited on surfaces

3.1.3 Experimental Results

Biological surface loadings on ACMV components and the floor of the room indicates the potential of the ACMV system and resuspension from the room floor as bioaerosol sources. Bacterial and fungal loadings on the surfaces of four ACMV components are shown Figure 3.5 and Figure 3.6, respectively. In general, fungal loadings are significantly higher than bacterial loadings (by about an order of magnitude), which indicates that the surfaces of the ACMV system are more seriously polluted by fungi than bacteria. One contributing factor could be the higher deposition rate of airborne fungi onto surfaces due to their larger sizes, compared to airborne bacteria (Zhao and Wu, 2006). Another possible contributing factor is the difference in survivability between fungi and bacteria on surfaces of materials used in typical ACMV systems, for species commonly found in indoor environment (Batterman and Burge, 1995). Since this study employs culture-based method which can only reflect the culture-able portion of microorganisms, the survivability factor can be significant. However, due to the limitation of the culture-based method, evaluation of the impact of survivability factor is beyond the scope of this study.

The fan blade in the AC unit has significantly higher bacterial and fungal loadings than the other sampled components. The fan is immediately behind the return air filter with its blades occupying a large portion of the cross-section area of the return air path. As a result, it is possible that a considerable portion

of bioaerosols, which are not captured by the return air filter, deposit on the surfaces of the fan blades. Bacterial loading on the return air filter is only about 20% of that on the fan blade whereas fungal loading on the return air filter is about 40% of that on the fan blade. This suggests that the return air filter has much better filtration efficiency to airborne fungi than to airborne bacteria.

Figure 3.6 Bacterial concentrations on surfaces of components of the ACMV system. Error bars show the standard error of mean of 5 repeats for the sample of each surface sampling area.

Figure 3.7 Fungal concentrations on surfaces of components of the ACMV system. Error bars show the standard error of mean of 5 repeats for the sample of each surface sampling area.

The surfaces of the fresh air duct are having lower biological loading than surfaces in the return air path (fan blades and return air filter), which may be due to higher amount of bioaerosols passing through the return air path than the fresh air duct. Experimental results of a previous study conducted in Singapore show that indoor/outdoor ratio of airborne bacterial concentrations ranges from 2 to 5 and that of airborne fungal concentrations ranges from 0.4 to 3.5 (Rajasekar and Balasubramanian, 2011). In the room, return air flow rate (0.37

m³/s) is around 18 times more than the fresh air flow rate (0.02 m³/s), which means that the amount of airborne bacteria passing through the return air path is around 37 to 94 times more than that passing through the fresh air duct. The amount of airborne fungi passing through the return air path is around 6 to 65 times more than that passing through the fresh air duct. The differences in surface loadings of bacteria and fungi on the insulated flexible duct and galvanised steel duct are statistically insignificant ($p > 0.05$).

Biological surface loadings in the ACMV system suggest that ACMV system is a potential bioaerosol source. The surface loadings found in this study are of similar orders of magnitude as a previous study conducted in China (Lu et al., 2009), which reported a bacterial loading of 2.9×10^5 and a fungal loading of 1.4×10^6 CFU/m² on the surface of an air handling unit.

3.1.4 Conclusion

Surfaces of components in the fresh air path and return air path of the ACMV system are both polluted by bacteria and fungi. Among all the sampled surfaces, that of fan blades are found to be most seriously polluted compared to other sampled surfaces, and have the potential to be significant bioaerosol emission source. According to surface sampling results, ACMV system is more likely to be a fungal emission source than bacterial emission source due to the fact that

fungus loadings on the sampled surface is about one order of magnitude heavier than bacterial loadings.

3.2 Air Sampling of Indoor Bioaerosol Concentration

3.2.1 Introduction

Previous studies (Siegel and Nazaroff, 2003; Wu et al., 2016) have suggested that biological particles can deposit, accumulate, and some can subsequently multiply on the surfaces of various components of an ACMV system. Bioaerosols will be formed and carried into indoor space by the supply air provided by the ACMV system when air flow passes through the surfaces and the biological particles are suspended. In order to investigate bioaerosol emission from the ACMV system, most of the previous studies focused on the measurement and analysis of biological loadings on internal surfaces of ventilation ducts and external surfaces of cooling coils, which are conducted in temperate climate regions (Schmidt et al., 2012; Siegel and Carey, 2001; Zhao and Wu, 2006). Contribution of bioaerosol emission from the ACMV system to indoor bioaerosol concentrations is still not investigated in tropical indoor environment.

In Section 3.1, experimental results of bioaerosol loadings in the ACMV system indicate the potential of bioaerosol emission from ACMV system as an indoor bioaerosol source. This section investigates contribution of bioaerosol emission from an ACMV system to indoor bioaerosol concentrations in Singapore. A series of experiments employing air sampling methods to measure indoor airborne bacterial and fungal concentrations are conducted in the same tutorial room, where surface sampling experiments introduced in Chapter 3 are performed, to investigate the contribution of different bioaerosol sources including bioaerosol emission from the ACMV system to indoor bioaerosol concentrations and compare relative effectiveness of lowering indoor bioaerosol concentrations between natural ventilation and ACMV.

3.2.2 Experiment

3.2.2.1 Agar Preparation and Transportation

Agar preparation used for air sampling and analysis shares the same method with that used for surface sampling and analysis except that the agar plates are taken out from the refrigerator and put into a sterilized cool box (ESKY) 10 hours before experiment to allow gradual adjustment to room temperature, which can avoid condensation on the inside of the agar plates during the transportation from laboratory to sampling site. The agar plates are transported to the sampling site in the cool box. After sampling, the sampled agar plates are immediately sealed by the film (PM 996, PARAFILM) and stored in another

sterilized cool box to transport the agar plates back to the laboratory after a day of experiment.

3.2.2.2 Air Sampling of Bioaerosols

Time-varied indoor bioaerosol concentration was used to fit the indoor material-balance model in the later section. This was to quantify the contribution of different emanation sources to indoor bioaerosol concentration. Due to limitations in microscopic methods and DNA-based analysis that require time-integrated measurement with sampling period of hours [18], a culture-based method was employed for the biological analysis of this study. However, the culture-based method can only be applicable to living and cultivable microorganisms. Air sampling is conducted indoors using a 200-hole, one-stage impactor (Biostage, SKC) connected to a sampling pump (Flite 3, SKC) which provides a sampling flow rate of 14.15 L/min. Before each sampling experiment, the air sampling flow rate of the impactor with a dummy agar plate inside is calibrated by a calibrator (Defender 510, SKC) shown in Figure 3.8. After calibration, the dummy agar plate is discarded and the impactor is kept in the biological safety cabinet (NO.NU-440-400E, NUAIRE) under UV light for sterilization before it is transported to the sampling site. Between each sampling, the impactor is cleaned by laboratory wipes (Kimwipes, Kimberley-Clark) with disinfectant. After each day of experiment, the impactor is cleaned by

laboratory wipes with disinfectant and sterilized by exposure to UV light for at least two hours.

Figure 3.8 Calibration of flow rate of an impactor with a pump.

Incubation and colony counting of air-sampled agar plates shares the same method as that for surface sampling. The bioaerosol concentration (C , colony forming unit (CFU) per cubic meters) can be calculated as $C = 1000n / (F \cdot t_0)$, where n (CFU) is the number of colonies on a sampled agar plate. $F = 14.15$ L/min is the sampling air flow rate of the impactor with an agar plate inside. $t_0 = 10$ min is the sampling time for each sample. Overlap of bioaerosol spores on agar plates is a potential cause of uncertainties of experimental data using Biostage for air sampling. Therefore, correction factor, which is used to mitigate uncertainty of the sampling results, is applied in the colony counting results (Macher, 1989).

3.2.2.3 Indoor Bioaerosol Dynamics Model

In order to analyse the contribution of bioaerosol emission from the ACMV system to indoor bioaerosol concentration, it is necessary to understand indoor bioaerosol dynamics. The tutorial room is visually inspected to be free from potential bioaerosol sources such as damped area, rotted food or damaged furniture, which means that bioaerosol resuspension from the floor, infiltration of outdoor bioaerosols, bioaerosols emitted from the occupants and the ACMV system (the fresh air duct and the AC unit), and bioaerosol resuspension from the floor are considered as the only potential bioaerosol sources in the tutorial room. Bioaerosol deposition on sidewall and ceiling of the room can be ignored because aerodynamic diameters of predominant species of indoor airborne bacteria and fungi in Singapore are larger than 0.5 μm (2016a; 2016c; Foster, 1996; Yamamoto et al., 2014) and deposition of particles of this size range (aerodynamics diameters larger than 0.5 μm) is predominantly attributed to gravitational settling on the floor in the indoor space (Whyte and Derks, 2015). Bioaerosol resuspension from the sidewall and the ceiling can also be ignored because resuspension of particles from indoor surfaces are primarily caused by human activities in the indoor environment (You and Wan, 2015), where there are no human activities to induce particle resuspension from the ceiling and the sidewall in the air sampling experiments. Indoor bioaerosol dynamics of the experimental room can be illustrated in Figure 3.9.

Figure 3.9 Schematic diagram of indoor bioaerosol dynamics

Indoor bioaerosols are assumed to be well mixed, which is a widely-accepted assumption for indoor aerosol modelling analysis (Schneider and Kildes, 1999; Zhou et al., 2011). Coagulation of indoor bioaerosols is also neglected because the indoor bioaerosol concentration is too low for coagulation to be significant (Zhou et al., 2011). The tutorial room is visually inspected to be free from potential bioaerosol sources such as damped area, rotted food or damaged furniture. Therefore, infiltration of outdoor bioaerosols, bioaerosols emitted from the occupants and the ACMV system (the fresh air duct and the AC unit) are considered the only potential bioaerosol sources in the tutorial room. Before air sampling experiments, leakage of the room is sealed by tapes to minimize the penetration of outdoor bioaerosols to indoor space. Therefore, the contribution of penetration from outdoor bioaerosols through leakage of the

indoor space is assumed to be neglected. Thus, a material-balance equation of indoor bioaerosol dynamics can be given by

$$V \frac{dC_i}{dt} = -C_i Q_f - Q_r \eta_1 C_i + R_{floor} N_{floor} A_{floor} - V_d C_i A + \sum_{j=0}^3 E_j, \quad (3.1)$$

where C_i (CFU/m³) is the indoor bioaerosol concentration, E_0 (CFU/s) is infiltration rate of outdoor bioaerosols, E_1 (CFU/s) is bioaerosol emission rate of human occupants, E_2 (CFU/s) is bioaerosol emission rate of the AC unit, E_3 (CFU/s) is bioaerosol emission rate of the fresh air duct. V (m³) is the volume of indoor space. Q_f (m³/s) is the fresh air flow rate. η_1 is filtration efficiency of the return air filter inside the AC unit. The return air flow rate (Q_r) is measured to be 0.37 m³/s using a hot-wire anemometer (9545-A, TSI). R_{floor} (#/s) is the resuspension rate of bioaerosols from the floor indoors. N_{floor} (CFU/m²) is the bacterial or fungal loading on the floor of the tutorial room, which is measured during the surface sampling described in Section 3.1. V_d (m/s) is the deposition velocity of indoor bioaerosols. A (m²) is the floor area. The values are estimated based on the sizes of predominant bioaerosol species in Singapore. The Values of parameters (R_{floor} , V_d and η_1) used in Equation (1) are summarized in Table 4.1. These are estimated based on the sizes of predominant bioaerosol species in Singapore. Predominant indoor airborne bacteria are *Staphylococcus* (aerodynamic diameter 0.5 – 1 µm) (Foster, 1996), *Micrococcus* (0.5 – 3.5 µm) (2016a) and *Streptococcus* (0.5 – 2 µm) (2016c). The predominant indoor airborne fungi are *Cladosporium* (5.52 µm), *Penicillium* (5.07 µm) and *Aspergillus* (5.16 µm) (Yamamoto et al., 2014) in Singapore (2016b).

Aerodynamic diameters of these airborne bacteria are in the range of 0.5 to 3.5 μm and the aerodynamic diameters of these airborne fungi are in the range of 5.07 to 5.52 μm . These size ranges are used to estimate the parameter values in Table 4.1, based on the correlations given in (Lai, 2002; Thatcher and Layton, 1995; Zhou et al., 2011).

3.2.2.4 Experimental Scenarios

Seven scenarios are designed to investigate contribution of different bioaerosol sources to indoor bioaerosol concentrations and the relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV, as shown in Figure 3.10. In each scenario, indoor air sampling is conducted from 9:30 am to 4:30 pm on each day and is repeated for five days. Each air sampling lasts for 10 min (e.g., the sampling representing 9:30 am actually starts from 9:25 am and ends at 9:35 am). Each scenario repeats for 5 days. There is a 20-min interval between two successive samplings. Fifteen air samples are collected in each day and, thus, seventy five air samples are collected for each scenario. Before the start of each scenario, air exchange rate (AER) in the room is measured by the tracer gas decay method using CO_2 (99.99% purity, Air Liquide) as the tracer gas. The CO_2 concentration is measured by a CO_2 meter (CM-0212, CO2Meter) at an interval of 20 s.

In Scenario 1, there is no occupant in the room and a HEPA (High Efficiency Particulate Arresting) filter (H13 Hepa Megalam MD13, Camfil) is inserted at the connection between the fresh air duct and the AC unit to prevent outdoor bioaerosols and bioaerosols emitted from the fresh air duct from entering the room. Hence, besides resuspension from the room floor (which is a bioaerosols source in all scenarios), bioaerosol emission from the AC unit is the only bioaerosol source for the indoor space. Scenario 2 is mostly similar to Scenario 1 but with the addition of two occupants. In Scenario 3, the HEPA filter is moved to the fresh air intake to prevent outdoor bioaerosols from entering the room but it becomes possible for the bioaerosols emitted from the fresh air duct to enter the room. In Scenario 4, the HEPA filter is removed. This scenario is the closest resemblance of the actual operation condition of the room among the 4 scenarios. Contribution to indoor bioaerosol levels by the occupants' emission can be illustrated by comparing Scenarios 1 and 2. Comparing Scenarios 2 and 3 will reveal the contribution by emission from the fresh air duct to indoor bioaerosol levels. Contribution to indoor bioaerosol levels by infiltration of outdoor bioaerosols can be illustrated by comparing Scenarios 3 and 4.

Two additional scenarios (Scenario 5 and 6, as shown in Figure 3) are defined to further study the impacts of the AC unit on indoor bioaerosol levels. In Scenario 5, the AC unit is switched OFF and disconnected from the fresh air duct, i.e., fresh air is supplied directly to the room without going through the

AC unit. A standing fan (FDF-30J6, FARFALLA) is used to maintain indoor air mixing and thermal comfort for the occupants. The air flow rate of the fan is $0.35 \text{ m}^3/\text{s}$, which is similar to the return air flow rate of the AC unit ($0.37 \text{ m}^3/\text{s}$). On this basis, deposition velocity (V_d) and resuspension rate (R_{floor}) of bioaerosols in this scenario is assumed to be the same as in other scenarios under ACMV system (Scenario 1-4 and 6). Indoor temperature and relative humidity (RH) are monitored at interval of 30 s during the experiment in this Scenario (CM0212, CO2meter).

The AC unit could impact on indoor bioaerosol levels in two ways: 1) emission and 2) removal by the return air filter. In Scenario 1 – 4 the AC unit operates with the return air filter on, i.e., the AC unit is making a ‘net’ effect (combination of 1) and 2)) to indoor bioaerosol levels. Comparing Scenario 5 with Scenario 4 shall reveal the ‘net’ effect of the AC unit on indoor bioaerosol levels. An additional scenario, Scenario 6, is designed to further investigate the breakdown of these two ways. In Scenario 6, the conditions are almost the same as in Scenario 4 except that the return air filter in the AC unit is removed. The difference in indoor bioaerosol concentrations between Scenario 5 and Scenario 6 should reflect the effect of bioaerosols emitted from the AC unit without the removal effect of the return air filter.

In Scenario 7, the room is naturally-ventilated (the window is open and the AC unit is switched OFF) and the same standing fan as in Scenario 5 (same fan flow rate setting) is used to provide thermal comfort. Scenario 7 represents the condition of the room under natural ventilation. Comparing Scenario 7 and Scenario 4 should reveal relative efficacy of controlling indoor bioaerosol level between using natural ventilation and using an ACMV system.

Table 3.1 summarizes sources/sinks of indoor bioaerosols in each of these scenarios. All experiments of this study are conducted in the period of June to September in 2016 (Southwest Monsoon Season). Significance analysis (t-test) of differences in surface biological concentrations and indoor bioaerosol concentrations is conducted. $p < 0.05$ is used to imply significant differences.

Scenario 3

Scenario 4

Scenario 5

Scenario 6

Scenario 7

Figure 3.10 Experimental setup of 7 scenarios

Table 3.1 Details of indoor bioaerosol sources in the seven experimental scenarios

Sources / sinks of indoor bioaerosols						
	AER	Infiltration of outdoor bioaerosols E_0	Number of human occupants E_1	AC unit		Fresh air duct E_3
				Emission E_2	Filtration by RAF η_1	
Scenario 1	0.516	No	0	Yes	Yes	No
Scenario 2	0.516	No	2	Yes	Yes	No
Scenario 3	0.588	No	2	Yes	Yes	Yes
Scenario 4	0.69	Yes	2	Yes	Yes	Yes
Scenario 5 [#]	0.69	Yes	2	No	No	Yes
Scenario 6*	0.69	Yes	2	Yes	No	Yes
Scenario 7	1.63	Yes	2	No	No	No

[#]The AC unit is switched OFF and disconnected from the fresh air duct

*The filter in the AC unit is taken off.

RAF – return air filter, AER – air exchange rate (hr^{-1}).

Bioaerosol resuspension from the indoor floor happens in all the seven scenarios.

3.2.3 Experimental Results

Indoor temperature and Relative Humidity (RH) is shown in Table 3.2. Average indoor bioaerosol concentrations of the seven scenarios (as shown in Figure 3.10) are shown in Figure 3.11. The averages are taken from all air samples collected in 5 days (75 samples) for each scenario. Average indoor airborne bacterial concentration in Scenario 2 is about double of that in Scenario 1

whereas the difference in average indoor airborne fungi concentration between the two scenarios is insignificant ($p>0.05$). This suggests that bioaerosol emission from the occupants contributes significantly to the indoor airborne bacterial concentration but have minor impacts on the indoor airborne fungal concentration.

Table 3.2. Indoor temperature and RH in the seven scenarios.

	Temperature (°C) (mean $\pm$ std.dev)	Relative Humidity (%) (mean $\pm$ std.dev)
Scenario 1	24.3 $\pm$ 0.5	64.6 $\pm$ 2.3
Scenario 2	25.3 $\pm$ 0.3	64.8 $\pm$ 2.9
Scenario 3	25.3 $\pm$ 1.0	66.1 $\pm$ 3.5
Scenario 4	24.9 $\pm$ 0.1	66.0 $\pm$ 2.5
Scenario 5	28.0 $\pm$ 0.3	69.5 $\pm$ 2.40
Scenario 6	25.7 $\pm$ 0.1	68.0 $\pm$ 2.0
Scenario 7	27.5 $\pm$ 0.7	69.5 $\pm$ 2.8

The average indoor airborne bacterial and fungal concentrations in Scenario 3 increase significantly ($p<0.05$) compared to Scenario 2. Both the average indoor airborne bacteria and fungi concentrations in Scenario 2 are about two-third of their counterparts in Scenario 3, as shown in Figure 3.11, indicating the effect of the fresh air duct as a bioaerosol source in Scenario 3. This observation echoes the surface sampling results shown earlier that surfaces of the fresh air duct are loaded with bacteria and fungi, which can be originated

from deposition of incoming bioaerosols or growth of deposited microorganisms on the duct surfaces (Batterman and Burge, 1995; Bluysen et al., 2003). Subsequently, the microorganisms on surfaces of the fresh air duct could be resuspended and transported indoors (You and Wan, 2014; Zhou et al., 2011).

Figure 3.11 Average indoor bioaerosol concentrations. Error bars show the standard error of mean of 5-day samples.

The difference in average indoor airborne bacteria concentrations between Scenario 3 and 4 is statistically insignificant ($p > 0.05$). However, the average indoor airborne fungi concentration in Scenario 3 is only about one-third of that

in Scenario 4, a statically significant difference ($p < 0.05$). The results suggest that fresh air infiltration has minor impact to indoor airborne bacteria concentration whereas fresh air infiltration is a major (or even the dominant) contributor to indoor airborne fungi concentration. Similar findings are reported in previous investigations conducted in other geographical locations such as the USA (Adams et al., 2013) and Europe (Pastuszka et al., 2000).

Difference in the average indoor airborne bacterial concentration between Scenario 5 and Scenario 4 is statistically insignificant ($p > 0.05$). However, the average indoor airborne fungal concentration in Scenario 5 is 47% higher than that in Scenario 4, a statistically significant difference ($p < 0.05$). The results show that the AC unit has a significant net effect of removing indoor airborne fungi but has a minor effect on indoor airborne bacteria. This may be because the predominant species of indoor airborne fungi in Singapore are in the larger size range that can be more easily captured by the MERV-4 rated return air filter in the AC unit (Burroughs, 2005), compared to indoor airborne bacteria.

Both the average indoor airborne bacterial and fungal concentrations in Scenario 6 are significantly higher than that in Scenario 5 ($p < 0.05$), indicating the effect of bioaerosol emission from the AC unit (without the removal effect of the return air filter). One point to note is that the indoor air temperature

($28.01 \pm 0.29^{\circ}\text{C}$ (arithmetic mean $\pm$ std.dev)) and RH (69.52 ± 2.4) in Scenario 5 is higher than all other scenarios with AC unit on, which are mentioned in Section 2.1 and Section 2.5. This may impact the comparability between Scenario 5 and other scenarios. However, more in-depth investigation of the temperature and RH effect on indoor bioaerosol level is beyond the scope of this paper.

Significantly higher indoor airborne fungal concentrations and lower bacterial concentrations in Scenario 7 than those in Scenario 4, statistically ($p < 0.05$), indicates that natural ventilation is more effective at removing indoor airborne bacteria and less effective for airborne fungi than ACMV in the room. The differences may be due to absence of bacterial emission from the ACMV system and more infiltration of outdoor airborne fungi under natural ventilation compared to ACMV in the room, which will be further quantitatively analysed in the next Chapter.

3.2.4 Conclusion

Indoor occupants can contribute significantly to indoor airborne bacterial concentrations while have minor impacts on indoor airborne fungal concentrations. Among all the bioaerosol sources, infiltration of outdoor bioaerosols contributes most to the indoor airborne fungal concentrations. Both

the bioaerosol emission from the AC unit and the fresh air duct can contribute to indoor bioaerosol concentrations significantly, indicating their substantial effects as bioaerosol emission sources for the indoor space. The natural ventilation is more effective at lowering indoor airborne bacterial concentrations while less effective at lowering indoor fungal concentrations compared to ACMV.

3.3 Identification of Bioaerosol Species Emitted from the Air-Conditioning and Mechanical Ventilation System

3.3.1 Introduction

Identification of species of bioaerosols originated from the ACMV system is required to understand health effects due to bioaerosol emission from the ACMV system on indoor occupants. Over the years, species identification of bioaerosol species from ACMV system has been conducted by performing surface sampling and biological analysis of biological loadings on surfaces of components of ACMV systems in areas like China (Lu et al., 2009), Europe (Hussein et al., 2013) and the USA (Foarde et al., 1997). The knowledge of species profile of bioaerosol emission from ACMV systems in tropical indoor environment is still lacking. In this chapter, air sampling experiments are conducted to sample airborne bacteria and fungi emitted from the ACMV

system (the AC unit and the fresh air duct) and the air samples are sent for species identification by DNA-based technology. Experimental results of species of bioaerosol emitted from the ACMV system can serve as the basis to study health effects of bioaerosol emission from the ACMV system.

3.3.2 Experimental Method

3.3.2.1 Experimental Site

Air sampling of bioaerosols emitted from the AC unit and the fresh air duct is conducted in the same room as that for surface sampling experiments in Section 3.1 and air sampling of indoor bioaerosols introduced in Section 3.2.

3.3.2.2 Agar Preparation and Transportation

Preparation and transportation of agar plates used for air sampling and analysis of bioaerosol emitted from the ACMV system shares the same method with that for indoor bioaerosol sampling used in Chapter 4.

3.3.2.3 Air Sampling

To identify the bacterial and fungal species emitted from the ACMV system, air samplings are conducted in two settings, as shown in Figure 3.12. In the first setting (Figure 3.12 (a)), an impactor (sampler) is put at the end of the fresh air duct to collect bioaerosols emitted from the fresh air duct while a HEPA filter is

installed at the fresh air intake to remove outdoor bioaerosol infiltration. Therefore, only bioaerosols emitted from the fresh air duct can be collected by the sampler. In the second setting (Figure 3.12 (b)), the AC unit is disconnected from the fresh air duct. A chamber is connected to the return air grill of the AC unit. Pure nitrogen is introduced into the chamber to replace the room air as return air. With this set up, the return 'air' is free from bioaerosols from the room. A sampler is used to collect bioaerosols in supply air, which only carries bioaerosols emitted from the AC unit. Since the sampling is at high flow speed area and the main flow stream is not aligned with the sampling flow stream direction, a reducer is attached to the sampler inlet in order to satisfy isokinetic sampling condition.

Air sampling is conducted in batches of 12 samples in each batch. Each air sampling lasts for 10 minutes and there is a 20 minutes' interval between two sampling. After finishing a batch, the samples are sent back to the laboratory for incubation using the same method described in Section 2.3. Colonies on the incubated samples are analyzed based on their morphological characteristics using standard taxonomic keys. This process continues until no colony of new morphological characteristics is observed in the latest batch. This is to ensure that all possible species are included. It ended up collecting and analysing 5 batches for the first setting and 3 batches for the second setting. Colonies on the agar plates are marked at back of the agar plates according to their

morphological characteristics. These agar plates are then sent for species identification by the DNA sequencing method (Axil Scientific Pte Ltd, Singapore). PCR technology is used to obtain the information of types of the bioaerosol species. The genomic DNA (gDNA) of bacterial and fungal colonies on the sampled agar plates are firstly extracted and then PCR reaction is performed to obtain the PCR product, which is the internal transcribed spacer (ITS) (700bp) for fungi and 16s rRNA (1400bp) for bacteria. With that, they will undergo a gel run to obtain the desired band, which are used for identification of the bacterial or fungal species.

Figure 3.12 Experimental setup for species identification

3.3.3 Results and Discussion

In the bioaerosol samples collected from the fresh air duct (Figure 3.12 (a)), 9 species of bacteria and 27 species of fungi are observed. In the bioaerosol samples collected from the AC unit (Figure 3.12 (b)), 13 species of bacteria and 33 species of fungi are observed. Number of species in each genus is summarized in Table 3.3 for bacteria and Table 3.4 for fungi.

Species of bioaerosols emitted from the AC unit is more diverse than that from the fresh air duct. A possible reason is that bioaerosols from both indoors and outdoors can deposit in the AC unit whereas in the fresh air duct, only the outdoor bioaerosols can deposit. Bioaerosols emitted from the occupants can lead to higher diversity of the bioaerosols accumulated in the AC unit than that in the fresh air duct. It is worth noting that the species from the genera: *Bacillus*, *Staphylococcus*, *Corynebacterium*, *Moraxella*, *Aspergillus*, *Cladosporium* and *Penicillium* are potential pathogens (listed in Agri-food and Veterinary Authority of Singapore (AVA) and Ministry of Health in Singapore (2015)). Exposure to these species of bioaerosol are found to be potential cause of wound infection (Hanson et al., 1977), eyes and skin infection (Cromartie et al., 1947), blood infection (Kowalski and Harwick, 1986), allergy and asthma (Fergusson et al., 1984). Among them, the pathogenic species of the *Moraxella* family (well-known to be originated from human (Goldstein et al., 2009) is only observed in the samples collected from the AC unit. It indicates that

pathogens originated from occupants can accumulate in the ACMV system and subsequently become bioaerosol sources.

Table 3.3 Number of bacterial species emitted from the ACMV system

Genus	Fresh air duct	AC unit
Bacillus*	1	3
Corynebacterium*	0	2
Micrococcus	3	3
Moraxella*	0	1
Staphylococcus*	2	4
Streptomyces	3	0
Total number of species	9	13

*Listed potential pathogen in Agri-food and Veterinary Authority of Singapore (AVA) and Ministry of Health in Singapore (2015).

Table 3.4. Number of fungal species emitted from the ACMV system

Genus	Fresh air duct	AC unit
Anthracoystis	1	0
Aspergillus*	9	1
Cerrena	0	1
<u>Cladosporium</u> *	4	4
Coprinellus	0	1

Corioloopsis	1	0
Curvularia	0	2
Eurotiales	1	0
Fungal endophyte	2	0
Kalmanozyma	0	1
Letendraea	0	3
Microdochium	0	1
Moesziomyces parantarcticus	0	1
Paecilomyces	0	2
Penicillium*	5	5
Phanerochaete	1	4
Phlebiopsis	1	1
Pseudozyma	2	0
Purpureocillium	0	0
Roussoella	0	1
Talaromyces	0	2
Toxicocladorporium	0	1
Trametes	0	1
Ustilago	0	1
Total number of species	27	33

*Listed potential pathogen in Agri-food and Veterinary Authority of Singapore (AVA) and Ministry of Health in Singapore (2015).

3.3.4 Conclusion

Some species of bioaerosols emitted from the ACMV system are potential pathogen, which is harmful to human health. The pathogenic species of bioaerosols originated from human body is found to be emitted from the AC unit, indicating that some bioaerosols originated from indoor occupants can deposit and accumulate in the ACMV system and be emitted into indoor space.

Chapter 4 Analytical Study of Bioaerosol Emission from Air-Conditioning and Mechanical Ventilation System

4.1 Quantification of Emission Rate of Indoor Bioaerosol Sources

4.1.1 Introduction

Contribution of bioaerosol emission from the ACMV system can be quantified by bioaerosol emission rate. In addition, comparisons of emission rate for different indoor bioaerosol sources can contribute to understanding of relative contribution of different sources and serve as basis for further development of cleaning technologies for indoor bioaerosols.

4.1.2 Method

By employing Equation (3.1) as provided in Section 3.2, Indoor bioaerosols are assumed to be well mixed, which is a widely-accepted assumption for indoor aerosol modelling analysis (Schneider and Kildes, 1999; Zhou et al., 2011). Coagulation of indoor bioaerosols is also neglected because the indoor bioaerosol concentration is too low for coagulation to be significant (Zhou et al., 2011). Therefore, the infiltration of outdoor bioaerosols, and those emitted from the occupants and the ACMV system (the fresh air duct and the AC unit) are considered only potential source in the tutorial room. Before air sampling

experiments, leakage of the room is sealed by tapes to minimized penetration of outdoor bioaerosols to indoor space. Therefore, contribution of penetration of outdoor bioaerosols through leakage of the indoor space is assumed negligible.

The analytical solution of Equation (3.1) has the following form

$$C_i(t) = C_{i,1}(t) + C_{i,2}(t) + C_{i,3}(t) , \quad (4.1)$$

where $C_{i,1}$ indicates contribution of initial indoor bioaerosol concentration; $C_{i,2}$ denotes contribution of bioaerosol resuspension from the floor; $C_{i,3}$ is contribution of bioaerosol emission sources including occupants, the fresh air duct, the AC unit and infiltration of outdoor bioaerosols. $C_{i,1, 2, 3}$ could be fully expressed as:

$$C_{i,1}(t) = C_i(0)e^{-\frac{Q_f+Q_r\eta_1+V_dA}{V}t} , \quad (4.2)$$

$$C_{i,2}(t) = \frac{R_{floor}N_{floor}A_{floor}}{Q_f+Q_r\eta_1+V_dA} (1 - e^{-\frac{Q_f+Q_r\eta_1+V_dA}{V}t}) , \quad (4.3)$$

$$C_{i,3}(t) = \frac{\sum_{j=0}^3 E_j}{Q_f+Q_r\eta_1+V_dA} (1 - e^{-\frac{Q_f+Q_r\eta_1+V_dA}{V}t}) . \quad (4.4)$$

Resuspension rate (R_{floor}) and deposition rate (V_d) of indoor aerosols including bioaerosols from the floor can be assumed constant with time for simulating aerosol dynamics in the room, as reported in previous studies (Thatcher and Layton, 1995; Zhou et al., 2011). A list of parameters (R_{floor} , V_d and η_1) used in

the Equation (4.1) is summarized in Table 4.1. The parameters are estimated based the sizes of predominant bioaerosol species in Singapore. Predominant airborne bacteria indoors are *Staphylococcus* (aerodynamic diameter 0.5 – 1 μm) (Foster, 1996), *Micrococcus* (0.5 – 3.5 μm) (2016a) and *Streptococcus* (0.5 – 2 μm) (2016c). The predominant indoor airborne fungi are *Cladosporium* (5.52 μm), *Penicillium* (5.07 μm) and *Aspergillus* (5.16 μm) (Yamamoto et al., 2014) in Singapore (2016b). Aerodynamic diameter of these airborne bacteria is in a range from 0.5 to 3.5 μm and aerodynamic diameter of these airborne fungi is in a range from 5.07 to 5.52 μm . These size ranges are used to estimate the parameters in the material-balance equation based on the correlations given in Table 4.1 (Lai, 2002; Thatcher and Layton, 1995; Zhou et al., 2011).

Table 4.1 Specific parameters used in the model of indoor bioaerosol dynamics

	Bacteria	Fungi	reference
Indoor resuspension rate* (R_{floor} , 10^{-10}s^{-1})	33.74	230.6	(Zhou et al., 2011)
Indoor deposition velocity* (V_d , 10^{-5}m.s^{-1})	15.83	100.3	(Zhou et al., 2011)
Filtration efficiency of the return air filter* (η_1)	0.083	0.1067	(Burroughs, 2005)

*Applicable range of aerodynamic diameter (μm)-Bacteria: 0.5-3.5, Fungi: 5.07-5.52 (2016a; 2016c; Foster, 1996; Yamamoto et al., 2014).

In Equation (4.1), time t is the variable and E_j ($j = 0, 1, 2, 3$) are unknowns.

Experimental data of indoor bioaerosol concentrations in the first four scenarios

(Scenario 1 – 4, as shown in Figure 3.10 in Section 3.3) are used to determine E_j ($j = 0, 1, 2, 3$). The non-linear least squares method is applied to estimate the bioaerosol emission rates, E_j ($j = 0, 1, 2, 3$). Hence the air sampling data based on the solution (expressed by Equation (4.1)) are used to calculate E_j .

Calculation of bioaerosol emission rate by fitting the experimental results of indoor bioaerosol concentrations to mathematical model of indoor bioaerosol dynamics using non-linear least squares method can avoid the limitation of calculation of bioaerosol emission rate by resuspension model applied on surfaces in air path inside the ACMV system, that resuspension model is not applicable on surfaces of non-uniform distribution of bioaerosol loadings and non-flat surfaces. Bioaerosol emission rate of the ACMV system with non-uniform distribution of bioaerosol loadings on surfaces in its air path and non-flat surfaces in it can be calculated when the non-linear least squares method is applied, which can make bioaerosol emission from the ACMV system as an unknown “black box” and calculate the bioaerosol emission rate by impacts of the bioaerosol emission on indoor bioaerosol concentration without considering the complicated structure inside the ACMV system. Variance of analytical solution of indoor bioaerosol concentration and experimental results of indoor bioaerosol concentrations are described as H value in Equation (4.5). CE_i is experimental results of indoor bioaerosol concentrations. By minimizing H

value in Equation (4.5), sum of bioaerosol emission rates ($\sum_{j=0}^3 E_j$) can be calculated.

$$H = \sum_{i=1}^{15} \left(C_i \left(\sum_{j=0}^3 E_j, t \right) - C E_i \right)^2 \quad (4.5)$$

4.1.3 Results of Bioaerosol Emission Rates

Surface loading (N_{floor}) of bacteria and fungi on the floor are measured by the surface sampling method described in Section 3.1. The average bacterial loading on the floor is 2.53×10^5 CFU/m² and that of fungi is 2×10^4 CFU/m², which are used as N_{floor} in Equation (4.1). Bioaerosol emission rate of resuspension from the indoor floor can be calculated to be 3.1×10^{-2} CFU/m² for bacteria and 1.7×10^{-2} for fungi by biological loadings of bacteria and fungi on the floor, their corresponding resuspension rate shown in Table 4.1 and the floor area (36.54 m²).

Calculation of bioaerosol emission rate by fitting the experimental results of indoor bioaerosol concentrations to mathematical model of indoor bioaerosol using non-linear least squares method . When Equation (4.1) is applied to

Scenario 1¹ in Section 3.2, $E_0 = E_1 = E_3 = 0$. Therefore, the bioaerosol emission rate of the AC unit (E_2) becomes the only unknown parameter in Equation (4.1).

The analytical solution of equation (4.1) is used to fit the experimental indoor bioaerosol concentrations data in Scenario 1 by the non-linear least squares method. Emission rates of the AC unit (E_2) for bacteria and fungi are determined to be 0.85 CFU/s and 2.96 CFU/s, respectively. The experimental results of Scenario 1 and the fitted curve of Equation (4.1) are shown in Figure 4.1. p-value of the fitting for bacterial results is 0.23 and that for fungal results is 0.

Applying Equation (4.1) to Scenario 2², and using the E_2 determined from Scenario 1, $E_0 = E_3 = 0$. Bioaerosol emission rate of the occupants (E_1) is the only unknown parameter in the equation. Using the same fitting method for experimental results obtained in Scenario 2 (Figure 4.2), E_1 for bacteria and fungi are determined to be 1.1 CFU/s and 0.02 CFU/s, respectively. Since there are two occupants in the current experimental setting, it can be calculated that each occupant emits airborne bacteria at a rate of 0.55 CFU/s and fungi at a rate of 0.01 CFU/s. p-value of the fitting for bacterial results and fungal results are both 0.

¹ Scenario 1 is presented in Section 3.2.2.4

² Scenario 2 is presented in Section 3.2.2.4

One of previous studies showed that the emission rates of total (including both culturable and non-culturable) biological particles of a sitting person are $(14 \pm 14) \times 10^6$ /hr (arithmetic mean $\pm$ std.dev) for bacteria and $(14 \pm 21) \times 10^6$ /hr for fungi (Hospodsky et al., 2015). The ratio of culturable-to-total bacteria and fungi ranged from 9×10^{-4} to 9×10^{-2} and from 1.8×10^{-4} to 0.45, respectively (Nazaroff, 2014). The corresponding emission rate of the culturable ones from a sitting person should range from 0 CFU/s to 700 CFU/s for bacteria and from - 875 CFU/s to 875 CFU/s for fungi, which are well consistent with the findings of this work. It should be noted that the bioaerosol emission from occupants varies and depends on both the species and the extent of human activity (Nazaroff, 2014).

For Scenario 3³, $E_0 = 0$ and E_1 and E_2 determined from Scenario 1 and 2. The only unknown, bioaerosol emission rate of the fresh air duct (E_3), was determined by fitting non-linear equation with the experimental results. Emission rates of the fresh air duct can be calculated as 1.43 CFU/s and 0.87 CFU/s for bacteria and fungi, respectively. p-value of the fitting for bacterial results and fungal results are both 0.

³ Scenario 3 is presented in Section 3.2.2.4

After determining E_1 , E_2 and E_3 from the previous scenarios, E_0 (infiltration rate of outdoor bioaerosols) becomes the only remaining unknown in Scenario 4⁴ when Equation (4.1) is applied. E_0 is estimated to be 1.43 CFU/s for bacteria and 7.44 CFU/s for fungi. p-value of the fitting for bacterial results is 0.18 and that for fungal results is 0.

Figure 4.1 Daily time profile of indoor bioaerosol concentration (CFU/m³) in Scenario 1 (only bioaerosol emission from the AC unit) based on five days' average. Error bars show the maximum and minimum of five day samplings.

⁴ Scenario 4 is presented in Section 3.2.2.4

Figure 4.2 Daily time profile of indoor bioaerosol concentration (CFU/m³) in Scenario 2 (bioaerosol emission from AC unit and the occupants) based on five days' average. Error bars show the maximum and minimum of five day samplings.

Figure 4.3 Daily time profile of indoor bioaerosol concentration (CFU/m³) in Scenario 3 (bioaerosol emission from AC unit, the occupants and fresh air duct) based on five days' average. Error bars show the maximum and minimum of five day samplings.

Figure 4.4 Daily time profile of indoor bioaerosol concentration (CFU/m³) in Scenario 4 (bioaerosol emission from AC unit, the occupants, fresh air duct and infiltration of outdoor bioaerosols) based on five days' average. Error bars show the maximum and minimum of five day samplings.

The bioaerosol emission rates E_0 , E_1 , E_2 and E_3 , determined by experimental data fitting method are described in this section and the bioaerosol emission rates of resuspension from the floor are shown in Figure 4.5. The result shows that infiltration of outdoor airborne fungi is the dominant source of indoor airborne fungi. Emission rates of bacteria from the four sources in the current experimental setting are of similar magnitude. The occupant density in the current experimental setting is 18 m²/person, similar to the typical office environment of 20 m²/person as suggested in ASHRAE Standard 62.1-2010 (2010). However, for indoor environments with higher occupant densities, e.g., classrooms (2.9 – 4 m²/person), shopping malls (2.5 m²/person) (2010), it is expected that the bacteria emission from occupants will become the dominant source for indoor airborne bacteria.

The total bacteria emission rate of the ACMV system is 2.28 CFU/s and fungal emission rate is 3.83 CFU/s ($E_2 + E_3$). The higher emission rate of fungal particles indicates that the ACMV system is more likely to be fungal emission source than bacteria, which is consistent with the surface sampling results that the ACMV system is more polluted by fungi than bacteria. However, bacterial emission rate is higher than fungi of the fresh air duct. A possible reason is that many fungal particles emitted from the fresh air duct deposit on the AC unit as they are going through the 90-degree bend at the connection between the fresh air duct and the AC unit, as shown in Figure 3.3. Fresh air flow is horizontal when it passes through the fresh air duct, and turns 90 degree when it is combined with return air to form supply air. The deposition of bacteria emitted from the fresh air duct could be much less than fungi due to the smaller sizes of bacteria (Cong et al., 2017). Therefore, the fungal emission rate is less than the bacteria emission rate of the fresh air duct.

Scenario 7 has a significantly higher average indoor airborne fungal concentration ($p < 0.05$) and a significantly lower average bacterial concentration ($p < 0.05$) than Scenario 4. The results indicate that the ACMV system is more effective in controlling indoor airborne fungal level but less effective for bacterial level, compared to natural ventilation. A contributor to higher indoor airborne bacterial concentration under ACMV (Scenario 4) than natural ventilation (Scenario 7) should be the bioaerosol emission from the ACMV

system employed in the ACMV system. Higher AER in the natural ventilation system than ACMV system (more than double), which can lead to more infiltration of outdoor airborne fungi under natural ventilation, should be responsible for significantly higher indoor airborne fungal concentration in the natural ventilation system.

Figure 4.5 Estimated emission rates of bioaerosol from the sources.

4.1.4 Conclusion

By employing analytical solution of indoor bioaerosol concentration expressed by Equation (4.1) to fit the experimental data of indoor bioaerosol concentrations from Scenario 1 to 4 using non-linear least squares method, the

bioaerosol emission rates of different sources of indoor bioaerosols are calculated and compared. The airborne fungal emission rate is higher than the airborne bacterial emission rate of the ACMV system, indicating that the ACMV system is more likely to be a fungal emission source, which is consistent with the surface sampling results. Among all the bioaerosol sources in the room during the experiments, the infiltration of outdoor airborne fungi is found to be the predominant source of indoor airborne fungi (around double the sum of emission rates of other sources). In addition, the indoor occupants may contribute most to the indoor airborne bacterial concentration as compared to other sources when the occupants' density is high. Occupants' bacterial emission rate is much more significantly higher than their fungal emission rate, indicating that the occupants are more likely to be indoor bacterial sources.

4.2 Simulation of Mitigation of Indoor Bioaerosol Pollution by Removing Bioaerosol Emission from the Air-Conditioning and Mechanical Ventilation System

4.2.1 Introduction

The ACMV system in the current experimental setting is about 2 years old. The filters in the system have never been replaced/serviced. There was a major servicing/cleaning of the fresh air duct conducted about one year before the experiment. The observation that indoor bioaerosol concentrations are

significantly increased by bioaerosol emission from the ACMV system (in Chapter 3.2) and quantifications of the bioaerosol emission rate from the ACMV system (in Chapter 4.1) indicate that the bioaerosol emission from the ACMV system can be a significant source of indoor bioaerosols. Therefore, it is necessary to avoid bioaerosol pollution from the ACMV system by keeping ACMV system clean and develop effective cleaning technology of ACMV system. Investigation of mitigation of indoor bioaerosol pollution by cleaning the ACMV system can serve as a support for necessity of cleaning ACMV system and basis of development of ACMV cleaning technology. Due to difficulties in cleaning the ACMV system, analytical method is employed to investigate the mitigation of indoor bioaerosol pollution by removing bioaerosol emission from the ACMV system.

4.2.2 Method

In order to investigate the potential mitigation of indoor bioaerosol pollution by better cleaning of the ACMV system, three hypothetical cases are simulated to using Equation (4.1), which can describe indoor bioaerosol dynamics:

1. Bioaerosol emission of the fresh air duct is removed by setting $E_3 = 0$ (hypo 1).
2. Bioaerosol emission of the AC unit is removed by setting $E_2 = 0$ (hypo 2).
3. Bioaerosol emission of the ACMV system is removed by setting $E_2 = E_3 = 0$ (hypo 3).

The environmental parameters as listed in Table 4.1 in Section 4.1 are used in this simulation. AER, and other emission rates of Scenario 4 are used as inputs for the simulation. The fitting curve of scenarios 4 as shown in Figure 4.4 is used as a reference compared to the three hypotheses in the simulation. These inputs are summarised in Table 4.2. The three hypothetical cases are compared to the fitting of air sampling data obtained in Scenario 4 using Equation (4.1), which is used as the reference case in this comparison.

Table 4.2 Bioaerosol emission rates in the three modelling cases

		Bioaerosol emission rate (bacteria/fungi) (CFU/s)			
	AER	E ₀	E ₁	E ₂	E ₃
Hypo 1	0.69	1.32/7.12	1.1/0.02	0.85/2.96	0
Hypo 2	0.69	1.32/7.12	1.1/0.02	0	1.43/0.87
Hypo 3	0.69	1.32/7.12	1.1/0.02	0	0

4.2.3 Results and Discussion

The simulation results are shown in Figure 4.6. Compared to the reference case, the daily-integrated average indoor airborne bacterial concentration drop by 28% in hypo 1, 17% in hypo 2 and 45% in hypo 3. Compared to the reference case, the daily-integrated average indoor airborne fungal concentration drop by 8% in hypo 1, 26% in hypo 2 and 34% in hypo 3. The results indicate significant mitigation of indoor bioaerosol pollution when the bioaerosol emission from the ACMV system is removed. By comparing the time-dependent variation of

indoor airborne bacterial and fungal concentrations, it can also be found that the indoor airborne fungal concentration is around two hours earlier than the indoor airborne bacterial concentration to drop to a stable concentration. Previous experimental results of air sampling shows that ACMV is more effective in controlling indoor airborne fungal concentration but less effective in controlling indoor airborne bacterial concentration. When the bioaerosol emission from the ACMV system is removed, as shown in hypo 3, average indoor fungal concentration will drop to 97 CFU/m³ (stable concentration is 95 CFU/m³) and average indoor airborne bacterial concentration will drop to 51 CFU/m³ (stable concentration is 48 CFU/m³), which is around 30% less than the average indoor airborne bacterial concentration under natural ventilation in Scenario 7, indicating that ACMV can be more effective in lowering both indoor airborne bacterial and fungal concentration than the natural ventilation by cleaning the ACMV system. By comparing time-dependent variation of indoor airborne bacterial and fungal concentrations in the simulation, it can also be found that indoor airborne fungal concentration is around two hours earlier than the indoor airborne bacterial concentration to drop to a stable concentration. Previous experimental results of air sampling shows that ACMV is more effective in controlling indoor airborne fungal concentration but less effective in controlling indoor airborne bacterial concentration. When the bioaerosol emission from the ACMV system is removed, as shown in hypo 3, average indoor airborne bacterial concentration will drop to 51 CFU/m³, which is around 30% less than the average indoor airborne bacterial concentration under natural ventilation in

Scenario 7, indicating that ACMV can be more effective in lowering both indoor airborne bacterial and fungal concentration than the natural ventilation by cleaning the ACMV system.

Figure 4.6 Indoor bioaerosol concentrations (CFU/m³) in the three simulation cases and the reference case (fitting of Scenario 4).

4.2.4 Conclusion

Indoor bioaerosol concentrations can be effectively decreased by removing bioaerosol emission from the ACMV system according to the simulation results, indicating the effectiveness of cleaning ACMV system. In order to avoid the degradation of indoor air quality caused by the bioaerosol emission from the ACMV system, regular cleaning of the ACMV system is recommended to lower indoor bioaerosol concentrations. By cleaning the ACMV system, the

ACMV system can be more effective in lowering both indoor airborne bacterial and fungal concentration.

Chapter 5 Conclusions and Suggestion for Future work

5.1 Conclusions

In addition to thermal comfort, ACMV systems serve as an engineering means to control indoor air quality. However, The ACMV system has potential to contribute to indoor bioaerosols by emitting bioaerosols to indoor space. Therefore, whether the ACMV system is helping to control indoor air quality or is worsening indoor air quality remains debatable. This work aims at identifying the contribution of bioaerosol emission from an ACMV system to indoor bioaerosol concentrations in a tropical indoor environment, which serves as the basis for future indoor bioaerosol exposure estimation and to develop effective control measures against the bioaerosol emissions from ACMV systems.

Airborne bacteria and fungi are predominant bioaerosols indoors, which can cause serious diseases and are investigated in this work. In a mechanically-ventilated indoor space, an ACMV system can impact indoor bioaerosol dynamics in four aspects: 1) draw outdoor bioaerosols into indoor space through fresh air path by air exchange (Nazaroff, 2014), 2) remove indoor bioaerosols by exhausting indoor bioaerosols to outdoor space through exhaust air path by air exchange and filtering bioaerosols in the return air using the filter equipped in the ACMV system (Nazaroff, 2014), 2) distribute indoor

bioaerosols by controlling flow rate and direction of supply air (Hoge et al., 1994; Li et al., 2007; Menzies et al., 2000; Moser et al., 1979; Riley et al., 1962; Schulman and Kilbourne, 1962; Tang et al., 2006), 3) impact growth or decay of indoor bioaerosols by controlling indoor temperature and RH (relative humidity) (Chen and Hildemann, 2009; Górný et al., 1999; Pasanen et al., 2000). In addition, an ACMV system has the potential to be a bioaerosol emission source for indoor space.

In order to investigate bioaerosol emission from ACMV systems, most of the previous studies focused on the accumulation of microorganisms on internal surfaces of ventilation ducts and external surfaces of cooling coils (Schmidt et al., 2012; Siegel and Carey, 2001; Zhao and Wu, 2006) in temperate climate regions. Few studies explored the microorganism contamination surfaces of the ACMV system and their emissions to indoor spaces in areas under tropical climate, which make current understanding of potential of bioaerosol emission from ACMV system and its contribution to indoor bioaerosol concentration limited in these areas. Specifically, quantification and species-identification of bioaerosols emitted from the ACMV system need to be conducted in tropical indoor environment. In addition, comparison of relative effectiveness of controlling indoor bioaerosol concentrations between natural ventilation and ACMV is still not investigated in a tropical indoor environment, which makes it

difficult to evaluate applicability of natural ventilation as an alternative ventilation strategy of ACMV in these areas.

Surfaces of components in the fresh air path and return air path of the ACMV system are both polluted by bacteria and fungi. Among all the sampled surfaces, that of fan blades are found to be most seriously polluted compared to other sampled surfaces, and have the potential to be significant bioaerosol emission source. According to surface sampling results, ACMV system is more likely to be a fungal emission source than bacterial emission source due to the fact that fungal loadings on the sampled surface is about one order of magnitude heavier than bacterial loadings.

Indoor occupants can contribute significantly to indoor airborne bacterial concentrations while have minor impacts on indoor airborne fungal concentrations. Among all the bioaerosol sources, infiltration of outdoor bioaerosols contributes most to the indoor airborne fungal concentrations. Both the bioaerosol emission from the AC unit and the fresh air duct can contribute to indoor bioaerosol concentrations significantly, indicating their substantial effects as bioaerosol emission sources for the indoor space. The natural ventilation is more effective at lowering indoor airborne bacterial concentrations while less effective at lowering indoor fungal concentrations compared to ACMV.

By employing analytical solution of indoor bioaerosol concentration expressed by Equation (5.1) to fit experimental data of indoor bioaerosol concentrations from Scenario 1 to 4 using non-linear least squares method, bioaerosol emission rate of different sources of indoor bioaerosols are calculated and compared. Airborne fungal emission rate is higher than airborne bacterial emission rate of the ACMV system, indicating that the ACMV system is more likely to be a fungal emission source, which is consistent with the surface sampling results showing that the ACMV system is more polluted by fungi than bacteria. Among all the bioaerosol sources in the room during the experiments, infiltration of outdoor airborne fungi is found to be the predominant source of indoor airborne fungi (around double the sum of emission rates of other sources). In addition, indoor occupants may contribute most to indoor airborne bacterial concentration compared to other sources when the occupants' density is high. Occupants' bacterial emission rate is much more significantly higher than their fungal emission rate, indicating that the occupants are more likely to be indoor bacterial sources.

Indoor bioaerosol concentrations can be effectively decreased by removing bioaerosol emission from the ACMV system according to the simulation results, indicating effectiveness of cleaning ACMV system. In order to avoid degradation of indoor air quality caused by bioaerosol emission from the

ACMV system, regular cleaning of the ACMV system is recommended to lower indoor bioaerosol concentrations. By cleaning the ACMV system, the ACMV system can be more effective in lowering both indoor airborne bacterial and fungal concentration.

Some species of bioaerosols emitted from the ACMV system are potential pathogen, which is harmful to human health. The pathogenic species of bioaerosols originated from human body is found to be emitted from the AC unit, indicating that some bioaerosols originated from indoor occupants can deposit and accumulate in the ACMV system and be emitted into indoor space.

Compared to previous studies from literature, the experimental results of this study provide a direct demonstration and substantial support for contribution of bioaerosol emission from ACMV system to indoor bioaerosol concentrations, and contribute to understanding the role played by the ACMV system in indoor bioaerosol pollution. In addition, the scheme developed in this study provide an approach to quantify and species-identify bioaerosol emission from the ACMV system, which can be applied in real-site indoor environment as an effective tool for indoor bioaerosol exposure estimation and forms the basis to develop effective control measures against bioaerosol emanation from ACMV systems.

5.2 Suggestion for Future Study

Despite the above work accomplished, some more studies are still needed to complete the understanding of contribution of ACMV system to indoor bioaerosols and comparison of controlling effectiveness of indoor bioaerosols between natural ventilation and ACMV. In addition, technologies which can improve filtration efficiency of filters also have the potential to be effective approaches to remove indoor bioaerosols while reducing the energy consumption and are deserved to be further explored.

5.2.1 Impacts of Temperature and Relative Humidity on Comparison of Controlling Effects of Indoor Bioaerosol Concentrations between Natural Ventilation and ACMV

Indoor bioaerosol concentrations are associated with indoor environmental conditions. For example, growth and decay of indoor bacteria and fungi can be significantly affected by indoor temperature, RH (Ren et al., 1999), which is due to that most of bacteria and fungi species rely on specific environments to grow and propagate (Mentese et al., 2009). Indoor CO₂ level can be significantly associated with bioaerosol emission from indoor occupants, which is an important source of indoor bioaerosols, and accumulation of indoor bioaerosols due to the fact that it is an indicator of indoor occupants load and

ventilation rate, i.e. high CO₂ level indoors usually means high occupants load and ineffective ventilation rate of the indoor space (Mentese et al., 2012).

ACMV systems are widely used to provide cooling and dehumidification effects for indoor space under ACMV and can significantly affect the indoor environmental conditions. Therefore, impacts of cooling and dehumidification provided by ACMV systems for indoor space on indoor bioaerosol concentrations should be included to investigate contribution of ACMV systems to indoor bioaerosol concentrations. Absence of cooling and dehumidification effects by ACMV system for indoor space under natural ventilation will result in difference of its indoor environmental condition from that under ACMV, which should be taken into consideration for analysis of their relative effectiveness of controlling indoor bioaerosol concentrations. Further exploration of dehumidification and cooling effects by the ACMV system on indoor bioaerosol concentrations will contribute to completing our understanding of contribution of ACMV system to indoor bioaerosol concentrations and explaining the relative effectiveness in controlling indoor bioaerosol concentrations between ACMV and natural ventilation.

In addition to indoor environmental conditions, outdoor environmental conditions can affect indoor bioaerosol concentrations significantly by

impacting infiltration of outdoor bioaerosols, which contribute substantially to indoor bioaerosol concentrations, as demonstrated in this work already. Infiltration of outdoor bioaerosols is positively related to outdoor bioaerosol concentrations, which is mainly decided by outdoor environmental conditions, such as outdoor temperature, RH and rainfall. Therefore, infiltration of outdoor bioaerosols should vary with outdoor environmental conditions for both natural ventilation system and ACMV system. Comparison of relative effectiveness of controlling indoor bioaerosol level between ACMV and natural ventilation need be specified for different outdoor environmental conditions, for example, weather conditions, to figure out the situations when the natural ventilation is more effective in lowering indoor bioaerosol concentrations compared to ACMV.

5.2.2 Application of Acoustic Agglomeration for Improvement of Filtration Efficiency of the Filters in the Air-Conditioning and Mechanical Ventilation System

In building with ACMV system, filters inside the ACMV system predominantly serves to remove indoor bioaerosols and around 15% to 30% of the system energy consumption is devoted to air distribution, most of which is consumed by fans in the ACMV system to overcome losses (pressure drop) in the distribution ducts and across filters (Ng et al., 2017). The power that a fan spends on overcoming pressure drop of a filter could be calculated by:

$$E_f = \frac{Q\Delta p}{\eta_f \eta_m}, \quad (8.1)$$

Where E_f is fan power and Q is the airflow rate through the filter; Δp is the pressure drop across the filter; η_m and η_f are the stepper motor efficiency and fan efficiency, respectively. For typical parameters ($\eta_m = 0.9$, $\eta_f = 0.75$, $Q = 0.47 \text{ m}^3/\text{s}$ (Fisk et al., 2002)), the fan power required for MERV 3, 6, 11, 14 rating filter are shown by Figure 5.1. The pressure drop data is from Table 5.1 (Farr, 2013). According to Singapore Standard 553 (2016b), the use of fine dust filters of at least a rating of Minimum Efficiency Reporting Value (MERV) 14 is required in the event of poor outdoor air quality (such as that during haze events) or presence of indoor source of fine particles and recommended. As shown in Figure 5.1, energy consumption of a fan due to pressure drop of a MERV 14 filter is 5 times that of a MERV 3 filter whose filtration efficiency is significantly lower. As a result, it is necessary to develop approaches, which can have effective control of indoor bioaerosols while avoid significant energy consumption.

Table 5.1 MERV filter parameters

Standard 52.5 Minimum Efficiency Reporting Value	Composite Average Particle Size efficiency, % in size range			Average Arrestance, % by Standard 52.1 method	Minimum Final Resistance	
	Range 1 0.3 to 1.0	Range 2 1.0 to 3.0	Range 3 3.0 to 10.0		Pa	Inches of Water column
1	N/A	N/A	E3<20	$A_{\text{average}} < 65$	75	0.3
2	N/A	N/A	E3<20	$65 < A_{\text{average}} < 70$	75	0.3
3	N/A	N/A	E3<20	$70 < A_{\text{average}} < 75$	75	0.3
4	N/A	N/A	E3<20	$75 < A_{\text{average}}$	75	0.3
5	N/A	N/A	20<E3<35	N/A	150	0.6
6	N/A	N/A	35<E3<50	N/A	150	0.6
7	N/A	N/A	50<E3<70	N/A	150	0.6
8	N/A	N/A	70<E3	N/A	150	0.6
9	N/A	E2<50	85<E3	N/A	250	1.0
10	N/A	50<E2<65	85<E3	N/A	250	1.0
11	N/A	65<E2<80	85<E3	N/A	250	1.0
12	N/A	80<E2	90<E3	N/A	250	1.0
13	E1<75	90<E2	90<E3	N/A	350	1.4
14	75<E1<85	90<E2	90<E3	N/A	350	1.4
15	85<E1<95	90<E2	90<E3	N/A	350	1.4
16	95<E1	95<E2	95<E3	N/A	350	1.4

Figure 5.1 Fan powers of filters with different MERV rating

Acoustic agglomeration is the process in which the acoustic waves impose relative motion and collision among fine particles suspended in the gas media, leading to the formation of agglomerates. In a short period, the acoustic agglomeration can shift the size distribution of the particles from smaller sizes to larger ones significantly. For example, the increase of particles' average size could be as much as one order and two orders of magnitude, respectively (de Sarabia et al., 2003). Larger particles can be more easily captured by filters compared to particles of diameters from $0.1\ \mu\text{m}$ to $3\ \mu\text{m}$, as illustrated in Figure 5.2. Thus, it can enhance filtration effects of filters. Figure 5.2 shows typical variance of filter efficiency with respect to particle size. There is a U-shaped relationship between the filter efficiency and particle size. The filtration efficiency for smaller ($<0.01\ \mu\text{m}$) and larger particles ($>3\ \mu\text{m}$) is higher than

that for intermediate size particles (0.01 μm –3 μm). Smaller particles could be effectively captured by a filter due to the significant diffusion mechanism and larger particles will be filtered due to significant interception and inertial impaction. Neither the diffusion mechanism nor interception and inertial impaction could play a significant role for intermediate size particles (Liu and Wang, 1997), leading to lower filter efficiency. The wide installation of higher efficiency filters is closely related to the removal difficulty of the intermediate particles by lower efficiency filters.

Figure 5.2 Relationship between particle size and filtration efficiency of the filter (Farr, 2001).

Acoustic effects could shift the particle size distribution from smaller to larger sizes within short time. By shifting the size distribution of indoor bioaerosols to

larger sizes by acoustic agglomeration effects, the agglomerated indoor bioaerosols could be more easily captured by filters, which can make it possible to apply a filter of lower MERV rating without affecting removal efficiency of indoor bioaerosols. For example, the average increase of particle radius after one-second and five-second exposure to ultrasonic standing waves could be as high as one order and two orders of magnitude, respectively (De Sarabia and Gallego-Juarez, 1986). In previous experimental analysis, the mean particle diameter of an aerosol was increased by one order of magnitude by using acoustic agglomeration at low sound pressure levels (SPL) of 100 to 120 dB (Volk jr and Moroz, 1976), suggesting that the application of acoustic agglomeration in industrial processes was feasible. Future study will investigate acoustic agglomeration as an effective approach in ACMV systems for indoor bioaerosol control.

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