

# **Biological Agents - A Potential Occupational Hazard**

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**Abstract:** Bioaerosols are a mixture of agents like bacteria and their metabolic products, fungal toxins,  $\alpha$ -1,3-glucans, viruses, gases, vapors or fragments of biological origin. Several biological agents affect large occupational groups causing acute and chronic infections, parasitism, cancer, toxic and allergic reactions, etc. Respiratory symptoms and lung function impairments are the most widely studied bioaerosol associated health effects. Other occupations with abundant bioaerosol exposures include veterinarians, health care workers, workers of the food industry, cotton and flex industries, wood industry, metal working industry, paper industry, detergent industry, biotechnology workers, sewage workers, bakery workers, workers collecting and recycling waste. So far occupational exposure limit (OEL) and dose response relationships have not been established for most biological agents because of diversity of the particular agent exposure and the susceptibility of human beings to those biological agents (e.g., Genetic polymorphism). Further, methods to assess bioaerosol exposures prove to be of limited use in population studies. Therefore, extensive research is needed to establish better exposure assessment tools and to validate newly developed methods.

**Key words:** Bioaerosols, Microbial toxins, Infectious diseases, Respiratory diseases, Occupational exposure limit.

#### Introduction

Recently, scientific interest with respect to exposure to biological agents aroused due to a wide range of adverse health effects associated with them in occupational as well as residential indoor environment. As compared to chemical and physical agents, hazardous biological agents are less well defined. Several biological agents affect large occupational groups causing acute and chronic infections, parasitism, toxic and allergic reactions <sup>1</sup>. Most biological agents occur in the form of bioaerosols and rely upon air for transmission. Farming, cattle handling, swine confinement house, waste-water and sewage treatment are significant outdoor sources of bioaerosols. Indoor sources such as heating, ventilation and air conditioning (HVAC) systems,

doors, potable drinking water system, shoes, clothes etcrely upon humidity, temperature, nutrients, oxygen and light for the growth of microorganisms <sup>2,3</sup>.

### **Components of bioaerosols**

Bioaerosols are ubiquitous, highly variable, complex, natural or manmade in origin and contribute to about 5-34 % of indoor air pollution <sup>4</sup>. The American Conference of Governmental Industrial Hygienists (ACGIH) defines bioaerosols as being airborne particles consisting of living organisms, such as microorganisms [e.g., bacteria, fungi, viruses, gases, vapors or fragments of biological origin] of different sizes ranging from 0.3 to 100  $\mu$ m in diameter <sup>5,6</sup>. Bioaerosols also includes biochemical components of cell or spore

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walls or metabolic products of bacteria and fungi that remain even after the cell death in the form of endotoxins, mycotoxins,  $\alpha$ -1,3-glucans, which are potent allergens <sup>7</sup>.

Gram Negative bacteria such as Pantoea agglomerans produces endotoxins and it mainly occurs on freshly harvested grains, cotton bract, wood, hay and other plant materials. While Pseudomonas spp., Klebsiella spp., Rahnella spp. and Alcaligenes fecalis are present in the dust of plant origin<sup>8</sup>. Acinetobacter and Enterobacter spp. are found in the dust of animal origin while Escherichia coli, Campylobacter and Salmonella spp. are of animal fecal origin <sup>9,10</sup>. Aeromonas hydrophilia, Pseudomonas spp., Flavobacterium spp., Legionella pneumophilia <sup>11,12,13</sup>, Leptospira, Brucella are transmitted through contaminated water and infected animals via skin abrasions and mucus membranes in humans<sup>14</sup>.

Commonly found gram positive bacteria are Anthrobacter, Corynebacter, Brevi-bacterium, Microbacterium, Streptococcus, Staphylococcus spp., Bacillus subtilis, Bacillus anthracis, Saccharopolyspora rectivirgula, Micropolyspora faenia, Thermoactinomyces vulgaris, Thermoactinomyces thalophilus, Saccharomonospora viridis. Actinomycetes produce large numbers of very small airborne spores (1-3 µm diameter), which are potentially capable of penetrating into human lungs and are not easily expelled <sup>15,16,17</sup>.

Field fungi like *Alternaria* and *Verticillium* develops on grasses and fresh green waste <sup>18</sup>. Storage fungi like *Aspergillus, Penicillium, Eurotium, Trichoderma, Fusarium, Rhizopus* and *Mucor* grows on stored grains and plant materials. They produce mycotoxins like aflatoxins, ochratoxins, zearalenone, trichothecene and  $\alpha$ -1,3-glucan which are major component of bioaerosols.  $\alpha$ -1,3-glucan [fungal cell wall constituent] possess pro-inflamatory properties which causes chronic inflammation of lungs 17,18,19,20,21.

Viruses transmitted by the airborne route include SARS (Severe Acute Respiratory Syndrome) virus, enteric viruses of intestinal origin [produced at sewage treatment facilities], RSV (Respiratory Syncytial Virus), *Hantavirus*, *Varicella-Zooster* virus, Measles, Mumps and *Rubella* viruses <sup>22,23,24</sup>.

#### **Route of exposure**

Inhalation is the predominant route of exposure as most biological agents become airborne for their dispersal. Particle size is an important factor in determining the risk. Larger particles  $>20 \ \mu m$ e.g., fungal spores are deposited in the upper respiratory tract i.e. nose and nasopharynx, which are removed by sneezing, blowing of nose. Particles smaller than 20  $\mu$ m and >5  $\mu$ m are deposited in the larynx, trachea and bronchi. They are removed by mucocilliary actions and then swallowed. Particles  $<5 \,\mu m$  get deposited in the bronchioles and alveoli. They cannot be easily expelled out from the respiratory system, leading to various diseases <sup>25</sup>. Ingestion and Dermal contact are also the routes of exposure for bioaerosols.

Exposure of biological agents in various occupations during the various activities of the workers is shown in the Table 1 <sup>8,12,16,17,19</sup>.

# Health effects associated with bioaerosols

A number of bioaerosols may interact in complex ways to cause infection and inflammation to exposed individuals. Three major groups of disease associated with bioaerosols exposure includes i) infectious diseases, ii) respiratory diseases and iii) cancer.

#### i) Infectious diseases

Infectious diseases arise from bacteria, fungi, viruses, protozoa and helminthes. Infectious agents are transmitted from a reservoir to a susceptible host through direct contact, ingestion or inhalation (air borne transmission or vector borne transmission). Agricultural workers, veterinarians, health care workers (doctors, nurses, ward boys), laboratory workers (Microbiologist), biomedical workers studying infectious agents and pateints are more prone to infectious diseases.

# **Bacterial diseases**

Bacterial diseases such as tetanus, anthrax, brucellosis, leptospirosis, and tuberculosis are generally caused by neglecting minor wounds or

| <b>Biological agents</b>  | Occupational activities  |
|---|--|
| Gram-negative bacteria (GNB),<br>Storage fungi and Thermophilic<br>actinomycetes                              | Farmers - Harvesting, threshing, storing, haymaking and silo unloading   |
| Gram-positive bacteria, GNB & endotoxins, Actinomycetes   | Animal breeders - Handling, feeding and milking the animals  |
| Fungi & mycotoxins, GNB & endotoxins  | Agriculture Industries - Grain elevators   |
| Thermophilic actinomycetes<br>GNB & endotoxins<br>Gram-positive bacteria and their<br>enzymes e.g., a-amylase | Sugarcane industry - Storage of bales of bagasse<br>Cotton & flex industries - Processing of fibers<br>Food industries - Bread making, Sugar beet industry |
| GNB & endotoxins, Infectious microorganisms   | Poultry industries - Cleaning, spreading bed material, collecting eggs etc.  |
| GNB & endotoxins Filamentous<br>fungi   | Wood industry - Early stage-frame sawing, Late<br>stage-trimming & processing of wood chips  |
| GNB & endotoxins<br>GNB & endotoxins  | Metal working industry - Oil mist from coolant oil<br>Sewage treatment plant - Sludge treatment, Aeration<br>of tanks                                      |
| GNB & endotoxins, Filamentous<br>fungi  | Waste recycling - Waste sorting, Organic waste collection  |
| GNB- Pseudomonas,<br>Flavobacterium & Cvtophaga   | Print shop - Humidified droplet aerosols   |
| Immunotoxic products of fungi<br>and bacteria-enzymes and<br>endotoxins                                       | Biotechnology lab worker - Lab experiments,<br>Glassware washing   |
| Pathogenic organisms  | Health care workers (Doctors, Nurses, ward boys) -<br>Treatment of the patients  |
| Zoonotic diseases through infected animals  | Veterinarians - Handling of sick animals   |
| Aerosols of allergenic/toxigenic  | Librarians & Art renovators - Handling of damp,  |

# Table 1. Exposure of biological agents in various occupations 8,12,16,17,19

abrasions. Legionellosis and anthrax are transmitted through the inhalation of aerosolized *bacilli*. Though Anthrax has been almost eradicated from western countries, India is an endemic region for Anthrax because of unprotected livestock population. As per National Animal Diseases Referral Expert System (NADRES software accessible at www. nadres.res.in), Anthrax has been reported in 18 states of India during the last two decades (1991-2010) and a gradual decline has been found in the occurrence of Anthrax from 2002 onwards <sup>26</sup>. Brucellosis is an important zoonosis with a worldwide distribution. It is more prevalent in the western part of Asia, India, Middle Eastern, Southern European and Latin American countries. It has been prevalent in almost all states of India as 80 % of the population lives in villages in unhygienic condition and poverty <sup>27,28</sup>. The epidemiological data on this disease is incomplete due to the absence of proper laboratory facilities, lack of awareness and endemicity under-reporting as well as poor co-operation between veterinary and health services <sup>29</sup>. Leptospirosis is also considered as most common zoonosis in the world. The outbreaks of leptospirosis have been reported

from coastal Gujarat, Maharashtra, Kerala, Tamil Nadu, Andhra Pradesh, Karnataka and Andaman periodically <sup>30</sup>. According to Sethi et al. <sup>31</sup>, the incidence of leptospirosis increased in north India also. Farmers and animal husbandry staff are at higher risk in rural areas while outbreaks are reported due to flooding during monsoon in urban India <sup>32</sup>. Increased awareness amongst physicians about protean manifestations of leptospirosis, and early institution of antimicrobial therapy pending diagnostic confirmation will help to prevent mortality associated with complicated disease <sup>30</sup>. Table 2 shows the list of important bacterial pathogens, their route of transmission and diseases caused by them <sup>33,34,35</sup>.

# **Fungal diseases**

Airborne fungi such as *Aspergillus* and *Penicillium* causes respiratory infections <sup>36</sup>. Most infections, the commonest being Aspergillosis, can occur in immune-compromised hosts or as a secondary infection following inhalation of fungal spores <sup>37</sup>. Coccidiodis, Histoplasma and Blastomyces grow in soil or may be carried by birds is

linked to exposure through wind-borne or animalborne transmission.

### Viral diseases

Occupational viral diseases occur by animal respiratory viruses, poxviruses, enteroviruses and arboviruses. Infection may be acquired from the vector or from handling of animals or animal products in agriculture, clinical or autopsy specimens in laboratory, from aerosols or from glassware. In the work place, Hepatitis-B gets transmitted by infected blood and body fluids when they are in direct contact with the inside of the mouth, eye or with broken, scrapped or chapped skin. Exposure occurs through contaminated objects such as needle or scalpel. Other viral infections occurring among hospital workers are Human immune deficiency virus (HIV), Cytomegalovirus, Influenza, Varicella zooster virus, Herpes simplex virus-Type I & II 38. According to CDC <sup>39</sup> and ECDC <sup>40</sup>, airborne transmission of influenza-A H1N1 viruses have had an enormous impact on transportation industries and health-related businesses during the

Table 2. List of important bacterial pathogens <sup>33,34,35</sup>

| Bacterium                          | Transmission routes          | Disease   |
|------------------------------------|------------------------------|---|
| Bacillus anthracis                 | Inhalation                   | Cutaneous, pulmonary, or<br>gastrointestinal, Anthrax |
| Brucella spp.                      | Direct contact, food,        | Brucellosis   |
| inhalation                         |                              |   |
| Campylobacter jejuni               | Food, water, direct contact  | Campylobacterioses                                    |
| Clostridium botulinum              | Food                         | Botulism  |
| Clostridium perfringens            | Food, wounds                 | Gastroenteritis, gas gangrene                         |
| Coxiella burneti                   | Inhalation (infected dust),  | Q fever   |
|                                    | direct contact               |   |
| Enterohemorrhagic Escherichia coli | Food, water                  | Hemorrhagic colitis,                                  |
|                                    |                              | hemolyticuremic syndrome                              |
| Leptospira spp.                    | Direct contact, skin lesions | Leptospirosis   |
| Listeria monocytogenes             | Food, water, inhalation      | Listerosis  |
| Mycobacterium bovis and            | Inhalation, undercooked      | Tuberculosis  |
| tuberculosis                       | food, skin wounds            |   |
| Salmonella spp. (nontyphoidal)     | Food, fomites, water         | Salmonellosis, acute                                  |
|                                    |                              | gastroenteritis, Guillain-Barré                       |
|                                    |                              | syndrome  |
| Yersinia enterocolitica and        | Food, direct contact, water  | Yersiniosis   |
| pseudotuberculosis                 |                              |   |

worldwide outbreaks of H1N1 flu. List of important viral diseases and their causative agents are given in Table 3.

# Parasitic diseases

Parasitic infections are generally caused by Protozoa, Helminthes and Arthropods. Certain occupational groups such as ditch workers, plumbers, lifeguards, sewer workers, recreation workers are at high risk of creeping eruption, hookworm diseases and Ascariasis. This might be because of their direct contact with the infective form of a parasite and indirectly due to inadequate sanitation and hygiene <sup>41</sup>. Table 4 shows the protozoal diseases and their causative agents.

# ii) Respiratory diseases

These diseases result from exposure to antigens which stimulate an allergic response in the body. Allergic response can range from acute mild conditions to severe chronic respiratory diseases. Occupationally related respiratory symptoms results from airway inflammation caused by specific exposure to toxins, pro-inflammatory agents or allergens. There are two types of respiratory diseases: Non-allergic symptoms reflect a non-immune-specific airway inflammation, whereas Allergic symptoms reflect an immune-specific inflammation, in which various antibodies like IgE and IgG can play a major role 5. Symptoms of non-allergic work related asthma is often referred to as asthma like syndrome or irritant induced asthma. It is highly prevalent in farmers and farm related occupations <sup>42</sup> and assumed to be caused by bioaerosol exposures <sup>43</sup>. The clinical expression of airway disease is influenced by a combination of components of bioaerosols, the dose and duration of exposure, as well as intrinsic differences in the host response to bioaerosols (genetic polymorphisms)<sup>44</sup>. Many of the components of bioaerosols like endotoxins from gram-negative bacteria, peptidoglycan and  $\alpha$ -1,3-glucans from fungi, plants and some bacteria <sup>38,45</sup> are pathogen associated molecular patterns (PAMPs) that bind to specific recognition molecules and activate innate immune pathways.

Table 3. List of important viral pathogens<sup>33,34,35</sup>

| Virus                             | Transmission routes                                | Disease                           |
|-----------------------------------|--|-----------------------------------|
| Hepatitis E virus<br>Hepeviridae/ | Fecal-oral, food or water, possible direct contact | Hepatitis                         |
| Picorna viruses                   | Direct contact, fomites, inhalation, water         | Foot-and-mouth                    |
| H1N1 virus                        | Direct contact, inhalation                         | Swine influenza                   |
| SARS corona virus                 | Inhalation   | Severe acute respiratory syndrome |
| Rabies virus                      | Saliva (broken skin and mucous membranes)          | Rabies                            |
| Vesicular stomatitis virus        | Insect vectors                                     | Vesicular stomatitis              |

| Га | ble | 4. | List | of | important | protozoal | pathogens <sup>33,34,35</sup> |
|----|-----|----|------|----|-----------|-----------|-------------------------------|
|----|-----|----|------|----|-----------|-----------|-------------------------------|

| Protozoan  | Transmission routes   | Disease   |
|--|---|---|
| Balantidiasis coli<br>Cryptosporidium parvum<br>Giardia lamblia<br>Microsporidia (many genera) | Food, water<br>Direct contact, food, water, inhalation<br>Food, water<br>Possible ingestion of dirty water, | Balantidiasis<br>Cryptosporidiosis<br>Giardiasis<br>Microsporidosis |
| Toxoplasmosis gondii   | Fecal-oral, water, undercooked meat   | Toxoplasmosis   |

Endotoxin-exposed workers are at an increased risk of non-atopic asthma and lung-function decline. Smit et al., <sup>46</sup> found association between occupational endotoxin exposure and wheeze in agricultural workers. Their study suggests that carriers of the functional CD14/-260C allele were more responsive to endotoxin exposure than T allele homozygotes due to significant modification by innate immunity genetic variants in CD14 and MD2. According to Dutkiewicz <sup>15</sup>, Bacteria and fungi occurring in organic dust may exert adverse effects on respiratory tract of exposed persons causing (a) Mucous membrane irritation [MMI] (b)Immunotoxic diseases [organic dust toxic syndrome (ODTS), inhalation fever, grain fever, silo unloader's disease, toxic pneumonitis], byssinosis, humidified syndrome, mycotoxicoses, sick building syndrome and (c) Allergic diseases such as allergic alveolitis [hypersensitivity pneumonitis (HP), granulomatous pneumonitis], asthma, allergic rhinitis.

Asthma and allergic rhinitis are the most extensively studied respiratory diseases associated with bioaerosol exposure. Diseases caused by agricultural organic dust exposure may result from periodic and short-term exposure to dust containing large number of microbes. Occupational endotoxin exposure has a protective effect on allergic sensitization and hay fever but increases the risk for ODTS and chronic bronchitis <sup>47</sup>. Disorders such as ODTS and HP occur at places where dust levels, microbial and endotoxin contents are very high e.g., 10-20 mg/m<sup>3</sup> dust level, 10<sup>6</sup>- 10<sup>9</sup> CFU/m<sup>3</sup> and 10<sup>3</sup>-10<sup>4</sup> EU/m<sup>3</sup> endotoxin content of air. Second common exposure pattern is daily exposure to a lower level of organic dust, e.g., 2-9 mg/m3 dust level, 103-10<sup>5</sup> CFU/m<sup>3</sup> microbial content and endotoxin concentration 50-900 EU/m<sup>3</sup>. Examples of such places are swine confinement units, a dairy barn or a poultry growing facility. Usual symptoms and disorders include those of acute and chronic bronchitis, asthma like syndrome and symptoms of MMI such as rhinitis 48.

#### Toxic gas exposure and respiratory diseases

During the processing of various agricultural products, researchers studied the presence of

various microorganisms such as thermophilic actinomycetes, gram negative bacteria with strong endotoxic and allergenic properties, different genera of fungi and their mycotoxins from airborne dust. High prevalence of the species *P. agglomerans* has also been detected <sup>49,50,51,52,53,54,55</sup>.

Agricultural workers face hazards from toxic gases produced by microorganisms. About 168 gaseous compounds have been identified in the air of livestock confinement building, where waste is stored in the deep system for more than several weeks <sup>56,48</sup>. Ammonia (NH<sub>2</sub>), hydrogen sulphide  $(H_2S)$ , methane  $(CH_1)$ , and carbon dioxide  $(CO_2)$ are most important gases produced from decomposing manure. Manure gases may always be present in low concentrations, when pits are agitated for pumping, some or all of these gases are rapidly released from the manure and may reach toxic levels or displace oxygen, increasing risk to humans and livestock. Among these, hydrogen sulfide (H<sub>2</sub>S) is considered as the most dangerous gas and found at the levels of above 100 ppm. Exposure to 100 ppm H<sub>2</sub>S produces pneumonitis, 500 ppm can be fatal in 30 minutes and concentrations over 700 ppm are rapidly fatal <sup>42,57</sup>. Ammonia (NH<sub>2</sub>) is commonly found gas in animal and poultry confinement operations. It is very soluble and associated with upper airway irritation, sinusitis, chronic obstructive pulmonary condition (COPD) and MMI 58. Methane is produced at manure storage areas and it is dangerously explosive at concentration more than 5%. Carbon dioxide is produced by microbial digestion of manure. It can reach to lethal proportion in 6-8 hours if ventilation equipment fails <sup>59</sup>. Oxides of nitrogen are formed during the fermentation of silage, which cause silo filler's disease. Silo is the upright cylindrical structure used widely for the storage & preservation of animal feed. Farmers enter the silo to unload the silage to feed the animals. Nitrogen oxide gases begin to form within hours of filling the silo and Nitrogen dioxide (NO<sub>2</sub>) becomes the predominant gas. The concentration raises quickly reaching peak within 5-7 days with levels ranging from several hundred to several thousand ppm 48. The oxides of nitrogen are heavier than air and accumulating in highest concentrations just above the silage bed. The recommended safe level for eight-hour exposure is 3 ppm. As inspired NO<sub>2</sub> is hydrated in lungs, it produces nitrous & nitric acid, which cause inflammation of the lungs. Lower concentrations up to 50-100 ppm can lead to milder symptoms of eye irritation, cough, nausea, fatigue and laryngo/ bronchospasm. Continuous exposure can lead to worsening of symptoms. High concentrations (over 200 ppm) can cause immediate loss of consciousness and the development of pulmonary edema in 12-24 hours. Brief exposure to high concentrations can lead to pulmonary edema and eventually bronchiolitis obliterans (irreversible pulmonary fibrosis) within weeks to months <sup>60</sup>.

# iii) Cancer

Certain oncogenic viruses and other biological agents cause cancer to exposed person <sup>5</sup>. Mycotoxins are the only clearly established non-viral biological occupational carcinogens. Aflatoxin, a mycotoxin from Aspergillus flavus, is the bestknown established human carcinogen capable of causing liver cancer 61,62,63. Ochratoxin A from A. ochraceus is also a possible human carcinogen. Ingestion is the predominant route of exposure to aflatoxin and ochratoxin A but exposure can also occur by inhalation in industries such as peanut processing, live stock feed processing or industries where grain dust exposure occurs 62,63,64,65. Live stock feed processing workers have an increased risk of liver cancer, cancer of biliary tract, salivary gland and multiple myeloma 66. Farmers are at increased risk for certain specific cancers, including hematological, lip, stomach, prostrate, connective tissue and brain cancer 67,68. Several studies have found associations between exposure to wood dust and various cancers in particular sinonasal cancer in furniture making and in other wood related jobs including cabinet making, carpentry and saw mills 69.

# **Evaluation of bioaerosols**

Monitoring of bioaerosols is a rapidly emerging area of industrial hygiene. The goal of monitoring is to obtain a representative sample of microorganisms present within the air that we breathe. Bioaerosol monitoring includes the measurement of viable and non-viable microorganisms in both indoor (e.g., industrial, office or residential) and outdoor (e.g. agricultural and general air quality) environment <sup>5,6,25</sup>. Viable bioaerosol sampling involves collecting bioaerosol and culturing the collected particulate. Only culturable microorganisms are enumerated and identified. Nonviable microorganisms are collected on a "greased" surface or a membrane filter. The microorganisms are then enumerated and identified using microscopy, classical microbiological or immunochemical techniques<sup>70</sup>.

The choice of the sampling method, in terms of air flow rate and the duration of sampling is based on the extent of the loads of bioaerosols, however there are no internationally accepted recommendations on sampling flow rate and the media used for sampling. Three common sampling techniques namely impaction, filtration and impingement are used to separate and collect the bioaerosols. Each of these methods, pulls a measured volume of air with the aid of an electric or battery operated pump<sup>71</sup>.

#### Identification/Quantification

Microorganisms have been quantified by convectional culture based techniques for indoor bioaerosol, in which colony forming units (CFU) on selective media are counted. From the corrected count and the sampling volume used, the number of colony forming units per cubic meter (CFU/m<sup>3</sup>) of air can be determined <sup>72</sup>. Various sampling methods and identification techniques are shown in Fig. 1 <sup>73</sup>.

Culture based methods are known to underestimate environmental microbial diversities. They also needs relatively long incubation time and are laborious. Therefore, non-culture based methods, such as immunoassays, molecular biological test, optical and electrical methods have been developed over the past few decades <sup>74</sup>. These includes Polymerase Chain Reaction (PCR)<sup>75</sup>, quantitative polymerase chain reaction (qPCR)<sup>76</sup>, microarray (Phylochip)<sup>77</sup>, fluorescent insitu-hybridization (FISH), flow cytometry<sup>78</sup>, solid phase cytometry <sup>79</sup> and immunoassay (ELISA)<sup>80</sup>.

PCR allows the detection and identification of non-culturable airborne microorganisms but it does



**Fig. 1.** Flow chart indicating selected examples of fungal and bacterial bioaerosols sampling methods and identification techniques in relation to sample processing (i.e., cultivation or non-cultivation)<sup>73</sup>

not distinguish between non-viable and viable microorganisms<sup>81</sup>. Currently, the real-time PCR (RT-PCR) is evolving into a promising tool capable of reproducible and accurate measurements of total microorganism concentrations in environmental samples. The advantage of RT-PCR is the capacity of rapid sample quantification as well as species-specific identification <sup>76</sup>. Thus, molecular biological methods are significantly faster and more sensitive than conventional culture based methods and they are also able to reveal a larger diversity of microbes. ATP bioluminescence is a fast, robust easy to perform and affordable method based on a light generating reaction with luciferin and firefly luciferase. This method detects both cultivable and non-cultivable organisms 82.

Spectroscopic techniques such as matrix assisted laser desorption/ionization time of flightmass spectrometry (MALDI-TOF-MS) or Raman-spectroscopy has been recently introduced for the analysis of bioaerosols. However, some of them are still depending on cultivation prior to investigation. Using instrumental improvements it is even possible to obtain a mass spectrum of a single airborne particle, allowing on-line measurements and analysis without the need of prior cultivation <sup>83</sup>.

### Control measures for reducing bioaerosols

In order to prevent or reduce occupational disease due to bioaerosols, certain control measures can be followed. These include reduction of dust at work place by effective ventilation and exhaust systems <sup>17</sup>, proper identification and elimination of the microbial source in the occupational environment, maintenance of equipments, humidity control, natural ventilation, use of filters in ventilation, and air cleaning by the use of disinfectants and biocides. Air in the operating rooms and other critical areas like isolation rooms can be disinfected by fumigation using microbicidal agents. Proper storing of the plant raw materials (grain, hay) at low temperature and humidity is also of great importance that prevents growth of microorganisms and overheating. Health education and creation of awareness about biological agents and periodic medical examination of the workers

exposed to organic dust is also very important for the control of biological hazards <sup>5,6,15,84</sup>.

# Risk assessment and occupational exposure limits (OEL)

Risk assessment presents many difficulties and is also influenced by the possible interaction of microorganisms with non-biological agents in the working environment and other factors acting on the same target organs. But the mechanisms operating simultaneously are not known. Risk assessment of bioaerosol-exposed workers is complicated by the diversity of agents in occupational environments and by few occupational exposure limits (OELs) set by regulatory organizations. Economic development and advanced technologies significantly influence biological risk.

There is an increasing need for OELs for bioaerosols such as endotoxins, fungal spores,  $\beta$ -1,3-Glucans, mycotoxins, allergens and enzymes that are known to exacerbate adverse health effects. More specific OELs are required as the complex composition of bioaerosols represents a major challenge for assessing risks.

Currently, neither OEL nor dose-response relationships have been established/available for most biological agents. Gram-negative bacteria endotoxin and  $\beta$ -1,3-Glucans are biologically derived agents under study by bioaerosols committee of ACGIH 5. Published values for acceptable bacterial and fungal bioaerosol concentrations vary from country to country. Health-based exposure limits have been proposed for endotoxins and fungal spores that are recommended for improved risk assessments. In the Netherlands, 90 endotoxin units/m<sup>3</sup> has been proposed as the OEL for endotoxins on the basis of acute respiratory effects 85. A lowest observed effect level (LOEL) of 100,000 spores/m<sup>3</sup> for nonpathogenic and non-mycotoxin producing fungal species has been proposed by Eduard in 2006 in a criteria document based on inflammatory respiratory effects 86.

ACGIH <sup>87</sup> stated that a general threshold limit value for culturable or countable bioaerosol concentrations is not scientifically supported due to the lack of exposure-response relationship data. According to ACGIH, bioaerosols in occupational settings are generally complex mixtures of various microbial, animal and plant particles. It has been difficult to establish exposure limits of bioaerosols since there is no uniform standardized method available for collection and analysis of bacterial and fungal biaerosols <sup>87,88</sup>.

# **Research needs**

National standards are unavailable for levels of airborne microorganisms and other biological agents. Since all microbial species are not alike, a dose response relationship has not been established for most biological agents. There is a lack of valid methods to assess quantitative exposure. Because of which one can't make out why minimal risks is caused by relatively high levels of one microbe or immediate action is triggered by modest or even extremely low levels of other microbes. Thus, this triggers a need for development and validation of the proper method for quantitative measurements of bioaerosols. Also, there is a need for further development of existing methods to make them more suitable for industrial hygiene purposes and large scale epidemiological studies.

### Conclusions

Bioaerosol exposure assessment is a rapidly evolving field. Till now, no sufficient OELs have been derived from organic dust risk assessment in the workplace. For proper indoor environment, there must be programmed and periodical cleaning operations. Proper maintenance activities as well as increase in the ventilation rate can play a role in improving the indoor air quality. The combination of the indoor microbial load data alongwith data from various studies related to health effects caused by inhalation of specific airborne microorganisms will allow the evaluation of various risks to which inhabitants are exposed.

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