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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – Air & Water Microbiology – SMB2203

1. General introduction

Aeromicrobiology is the study of living microbes which are suspended in the air. These microbes are referred to as bioaerosols. Though there are significantly less atmospheric microorganisms than there are in oceans and in soil, there is still a large enough number that they can affect the atmosphere. Once suspended in the air column, these microbes have the opportunity to travel long distances with the help of wind and precipitation, increasing the occurrence of widespread disease by these microorganisms. These aerosols are ecologically significant because they can be associated with disease in humans, animals and plants. Typically microbes will be suspended in clouds, where they are able to perform processes that alter the chemical composition of the cloud, and may even induce precipitation.

There are many factors within the physical environment that affect the launching, transport and deposition of bioaerosols. Particles which become suspended in the air column arise mainly from terrestrial and aquatic environments and are typically launched by air turbulence Winds are the primary means of transport for bioaerosols. Bioaerosols can be deposited by a number of mechanisms, including gravity pulling them down, making contact with surfaces, or combining with rain which pulls the particles back down to earth's surface.

Atmosphere:

Along with water droplets, dust particles and other matter, air contains microbes (Al-Dagal 1990). Microbes follow a particular pathway in which they are suspended into the atmosphere. First they are launched into the air. The source of the launching of airborne microbes stems from humans, animals and vegetation. then they are transported (by various methods including winds, machinery and people) and finally are deposited somewhere new. The atmosphere can have a variety of physical characteristics, and can be very extreme in terms of the relative humidity, temperature and radiation. These factors play a huge role in what kinds of microbes will survive in the atmosphere and how long they will stay alive.

Clouds:

One area that bioaerosols can be found in is within clouds. Cloud water is a mixture of organic and inorganic compounds suspended within moisture (contribution of microbial activity yo clouds). The conditions in clouds are not conducive to much life, as microbes present there must withstand freezing temperatures, the threat of desiccation, and extreme UV rays. Clouds are also an acidic environment, with a pH ranging from 3 to 7. Nevertheless, there are extremophile microbes which can withstand all of these environmental pressures. Clouds serve as a transport for these microbes, dispersing them over long distances.

Physical Environment Stresses:

The atmosphere is a difficult place for a microbe to survive. Dessication is the primary stress that aeromicrobes face, and it limits the amount of time that they can survive while suspended in the air (Pepper 2011). Humidity within the air is a second factor which can affect the survival of organisms. Certain bacteria, including Gram + bacteria, are more tolerant of high humidity in the air, while others are more tolerant of dessication and dry conditions, such as Gram + cells (Pepper 2011). Temperature must be in an intermediate range, as too hot of temperatures can denature proteins, and too cold of temperatures can cause ice crystal formation (Pepper 2011). Finally, radiation poses a potential hazard for aeromicrobes, as it can damage DNA within the cells.

II. Microbial Communities

Many different microorganisms can be in aerosol form in the atmosphere, including viruses, bacteria, fungi, yeasts and protozoans. In order to survive in the atmosphere, it is important that these microbes adapt to some of the harsh climatic characteristics of the exterior world, including temperature, gasses and humidity. Many of the microbes that are capable of surviving harsh conditions can readily form endospores, which can withstand extreme conditions. Many of these microorganisms can be associated with specific and commonly known diseases.

Bacterial

One such bacterial microorganism that can resist environmental stresses is *Bacillus anthracis*. It is a gram positive rod shaped bacteria that utilizes spore formation to resist environmental stresses. The spore is a dehydrated cell with extremely thick cell walls which can remain inactive for many years. This spore makes Bacillus anthracis a highly resilient bacteria, allowing it can survive extreme temperatures, chemical contamination, and low nutrient environments (Gatchalian 2010). This bacteria is associated with Anthrax, which is a severe respiratory disease that infects humans.

Fungal

Another such microorganism that can resist environmental stresses is *Aspergillus fumigatus*, which is a major airborne fungal pathogen (McCormick 2010). This pathogen is capable of causing many human diseases when conidia are inhaled into the lungs. While A. fumigatus lacks virulence traits, it is very adaptable to changing environmental conditions and therefore is still capable of mass infection. (McCormick 2010).

Viral

An example of a viral airborne pathogen is the Avian Influenza Virus, which is a single stranded RNA virus that can infect a broad range of animal species as well as humans and cause the Avian Influenza.

III. Microbial Processes

The figure on the bottom right depicts the processes that a microbe undergoes during its life cycle. The microbes undergo the emission process, in which they are emitted from surfaces such as water, soil or vegetation and become airborne and transported into the airstream. The red boxes indicate some of the harsh environmental conditions that the microbes must withstand while airborne. The microbes that are able to withstand and survive these environmental pressures are the more resistant varieties. The microbes make it into clouds, where they can begin the breakdown of organic compounds. Finally, the microbes are "rained" out of the clouds through wet deposition, and they begin colonization of their new location (Amato 2012).

Droplet Formation

The emission process mentioned above, in which microbes are lifted in the air often involves microbes being suspended in droplets, which are large enough to keep the microbes hydrated and large enough to maintain a virulent amount of pathogen, but are still small enough to stay suspended in the air (Robinson 2012).

Types of airborne diseases

Many diseases are spread through the air, including these:

01. **Coronavirus and COVID-19:** A rapidly spreading coronavirus, SARS-CoV-2, and the disease it causes, COVID-19, continue to cause widespread concern as of early 2020. Information on coronavirus and COVID-19 is constantly being updated as a result. The most common symptoms of COVID-19 include fever, cough, fatigue, and shortness of breath. If you experience these symptoms, see a doctor immediately.

02. **The common cold:** Millions Trusted Source of cases of the common cold occur each year in the United States. Most adults get two or three colds a year. Children tend to get them more frequently. The common cold is the top reason for absences at school and work. There are many viruses that can cause a cold, but it’s usually a rhinovirus.

03. **Influenza:** Millions Trusted Source of cases of the common cold occur each year in the United States. Most adults get two or three colds a year. Children tend to get them more frequently. The common cold is the top reason for absences at school and work. There are many viruses that can cause a cold, but it's usually a rhinovirus. There are many strains of the flu, and they are constantly changing. That makes it difficult for your body to develop immunities.

04. **Chickenpox:** Chickenpox is caused by the varicella-zoster virus. If you have chickenpox, you can spread it for a day or two before you get the telltale rash. It takes up to 21 days after exposure for the disease to develop. Most people get chickenpox only once, and then the virus goes dormant. Should the virus reactivate later in life, you get a painful skin condition called shingles. If you haven't had chickenpox, you can contract it from someone with shingles.

05. **Mumps:** Mumps is another very contagious viral disease. You can spread it before symptoms appear and for up to 5 days after. Mumps used to be quite common in the United States, but rates have declined by 99% Trusted Source due to vaccination. From January 1 to January 25, 2020, 70 cases in the United States were reported to the CDC. Outbreaks tend to occur in densely populated environments.

06. **Measles:** Measles is a very contagious disease, particularly in crowded conditions. The virus that causes measles can remain active in the air or on surfaces for up to 2 hours. You're able to transmit it to others up to 4 days before and 4 days after the measles rash appears. Most people get the measles only once. Measles is a leading cause of death among children worldwide and was responsible for 140,000 deaths (Trusted Source in 2018). It's estimated that the measles vaccine prevented around 23 million deaths from 2000 to 2018. The disease is less common in the United States and occurs mostly in people who haven't been vaccinated. There were 1,282 cases (Trusted Source reported in 2019). As of March 2, 2020, there have been 12 confirmed cases in 2020.

07. **Whooping cough (pertussis):** This respiratory illness causes swelling of the airways that results in a persistent hacking cough. It's at the height of contagiousness for about 2 weeks after the coughing starts. Worldwide, there are about 24.1 million Trusted Source cases of whooping cough every year, resulting in 160,700 deaths. In 2018 Trusted Source, there were 15,609 reported cases in the United States.

08. **Tuberculosis (TB):** TB, also known as consumption, is an airborne disease. This is a bacterial infection that doesn't spread easily. You generally have to be in close contact with a person who has it for a long time. You can contract TB without becoming ill or transmitting it to others. About 1.4 billion people worldwide have TB. Most aren't sick. About 10 million people worldwide have active TB. People with a weakened immune system have the greatest risk of developing the disease. Symptoms can appear within days of exposure. For some, it takes months or years to activate. When the disease is active, bacteria rapidly multiply and attack the lungs. It can spread through your bloodstream and lymph nodes to other organs, bones, or skin.

09. **Diphtheria:** Once a major cause of sickness and death in children, diphtheria is now rare in the United States. Due to widespread vaccination, fewer than five cases have been reported in the past decade. Worldwide, there were about 7,100 cases (Trusted Source of diphtheria in 2016), but it may be underreported. The disease injures your respiratory system and can damage your heart, kidneys, and nerves.

Symptoms

Airborne diseases usually result in one or more of the following symptoms:

01. inflammation of your nose, throat, sinuses, or lungs
02. coughing
03. sneezing
04. congestion
05. runny nose
06. sore throat
07. swollen glands
08. headache
09. body aches

10. loss of appetite
11. fever
12. fatigue

Chickenpox causes an itchy rash that usually starts on your chest, face, and back before spreading over the rest of your body. Within a few days, fluid-filled blisters form. The blisters burst and scab over in about a week.

The measles rash can take as long as 7 to 18 days to appear after you've been exposed. It generally starts on your face and neck, and then spreads over the course of a few days. It fades within a week.

Serious complications of measles include:

01. ear infections
02. diarrhea
03. dehydration
04. severe respiratory infection
05. blindness
06. swelling of the brain, or encephalitis

Whooping cough gets its name from its main symptom, a severe hacking cough, which is usually followed by a forceful intake of air. Symptoms of TB vary depending on which organs or body systems are affected and may include coughing up sputum or blood. Diphtheria can cause marked swelling in your neck. This can make it difficult to breathe and swallow. Complications from airborne diseases are more likely to affect the very young, the very old, and people with a compromised immune system.

Treatment for common airborne diseases

For most airborne diseases, you'll need plenty of rest and fluids. Further treatment depends on your specific illness. Some airborne diseases, such as chickenpox, have no targeted treatment. However, medications and other supportive care can help relieve symptoms. Some, such as the flu, can be treated with antiviral drugs. Treatment for infants with whooping cough can include antibiotics, and hospitalization is often needed. There are drugs to treat and cure TB, although some strains of TB are drug resistant. Failure to complete the course of medicine can lead to drug resistance and return of symptoms. If caught early enough, diphtheria can be successfully treated with antitoxins and antibiotics.

Incidence

Airborne diseases happen all around the world and affect virtually everyone. They spread easily in close quarters, such as schools and nursing homes. Large outbreaks tend to occur under crowded conditions and in places where hygiene and sanitation systems are poor. Incidence is lower in countries where vaccines are widely available and affordable.

Outlook

Most airborne diseases run their course within a few weeks. Others, like whooping cough, can last for months. Serious complications and longer recovery time are more likely if you have a weakened immune system or if you don't have access to good medical care. In some cases, airborne diseases can be fatal.

What you can do to prevent spreading an airborne disease

Although it's impossible to completely avoid airborne pathogens, there are some things you can do to lower your chances of getting sick:

01. Avoid close contact with people who have active symptoms of disease.
02. Stay home when you're sick. Don't let vulnerable people come in close contact with you.
03. If you must be around others, wear a face mask to prevent spreading or breathing in germs.
04. Cover your mouth when you cough or sneeze. Use a tissue or your elbow to cut down on the possibility of transmitting germs on your hands.
05. Wash your hands thoroughly (at least 20 seconds) and often, especially after sneezing or coughing.
06. Avoid touching your face or other people with unwashed hands.

Vaccines can reduce your chances of getting some airborne diseases. Vaccines also lower the risk for others in the community. Airborne diseases that have vaccines include:

01. chickenpox
02. diphtheria
03. influenza: vaccine updated every year to include strains most likely to spread in the coming season
04. measles: usually combined with vaccine for mumps and rubella, and is known as the MMR vaccine
05. mumps: MMR vaccine
06. TB: not generally recommended in the United States
07. whooping cough

In developing countries, mass immunization campaigns are helping to lower the transmission rates of some of these airborne diseases.

Air pollution

Air pollution occurs when harmful or excessive quantities of substances are introduced into Earth's atmosphere. Sources of air pollution include gases (such as ammonia, carbon monoxide, sulfur dioxide, nitrous oxides, methane and chlorofluorocarbons), particulates (both organic and inorganic), and biological molecules. It may cause diseases, allergies and even death to humans; it may also cause harm to other living organisms such as animals and food crops, and may damage the natural or built environment. Both human activity and natural processes can generate air pollution.

Air pollution is a significant risk factor for a number of pollution-related diseases, including respiratory infections, heart disease, COPD, stroke and lung cancer.[1] The human health effects of poor air quality are far reaching, but principally affect the body's respiratory system and the cardiovascular system. Individual reactions to air pollutants depend on the type of pollutant a person is exposed to, the degree of exposure, and the individual's health status and genetics. Indoor air pollution and poor urban air quality are listed as two of the world's worst toxic pollution problems in the 2008 Blacksmith Institute World's Worst Polluted Places report. Outdoor air pollution alone causes 2.1 to 4.21 million deaths annually. Overall, air pollution causes the deaths of around 7 million people worldwide each year, and is the world's largest single environmental health risk.

Productivity losses and degraded quality of life caused by air pollution are estimated to cost the world economy $5 trillion per year. Various pollution control technologies and strategies are available to reduce air pollution.

An air pollutant is a material in the air that can have adverse effects on humans and the ecosystem. The substance can be solid particles, liquid droplets, or gases. A pollutant can be of natural origin or man-made. Pollutants are classified as primary or secondary. Primary pollutants are usually produced by processes such as ash from a volcanic eruption. Other examples include carbon monoxide gas from motor vehicle exhausts or sulfur dioxide released from factories. Secondary pollutants are not emitted directly. Rather, they form in the air when primary pollutants react or interact. Ground level ozone is a prominent example of a secondary pollutant. Some pollutants may be both primary and secondary: they are both emitted directly and formed from other primary pollutants. Pollutants emitted into the atmosphere by human activity include: (CO_2) – Because of its role as a greenhouse gas it has been described as "the leading pollutant and "the worst climate pollutant" Carbon dioxide is a natural component of the atmosphere, essential for plant life and given off by the human respiratory system. This question of terminology has practical effects, for example as determining whether the U.S. Clean Air Act is deemed to regulate CO_2 emissions. CO_2 currently

forms about 410 parts per million (ppm) of earth's atmosphere, compared to about 280 ppm in pre-industrial times, and billions of metric tons of CO_2 are emitted annually by burning of fossil fuels. CO_2 increase in earth's atmosphere has been accelerating.

01. Sulfur oxides (SO_x) – particularly sulfur dioxide, a chemical compound with the formula SO_2. SO_2 is produced by volcanoes and in various industrial processes. Coal and petroleum often contain sulfur compounds, and their combustion generates sulfur dioxide. Further oxidation of SO_2, usually in the presence of a catalyst such as NO_2, forms H_2SO_4, and thus acid rain is formed.[2] This is one of the causes for concern over the environmental impact of the use of these fuels as power sources.

02. Nitrogen oxides (NO_x) – Nitrogen oxides, particularly nitrogen dioxide, are expelled from high temperature combustion, and are also produced during thunderstorms by electric discharge. They can be seen as a brown haze dome above or a plume downwind of cities. Nitrogen dioxide is a chemical compound with the formula NO_2. It is one of several nitrogen oxides. One of the most prominent air pollutants, this reddish-brown toxic gas has a characteristic sharp, biting odor.

03. Carbon monoxide (CO) – CO is a colorless, odorless, toxic gas.[21] It is a product of combustion of fuel such as natural gas, coal or wood. Vehicular exhaust contributes to the majority of carbon monoxide let into our atmosphere. It creates a smog type formation in the air that has been linked to many lung diseases and disruptions to the natural environment and animals.

04. Volatile organic compounds (VOC) – VOCs are a well-known outdoor air pollutant. They are categorized as either methane (CH_4) or non-methane (NMVOCs). Methane is an extremely efficient greenhouse gas which contributes to enhanced global warming. Other hydrocarbon VOCs are also significant greenhouse gases because of their role in creating ozone and prolonging the life of methane in the atmosphere. This effect varies depending on local air quality. The aromatic NMVOCs benzene, toluene and xylene are suspected carcinogens and may lead to leukemia with prolonged exposure. 1,3-butadiene is another dangerous compound often associated with industrial use.

05. Particulate matter / particles, alternatively referred to as particulate matter (PM), atmospheric particulate matter, or fine particles, are tiny particles of solid or liquid suspended in a gas. In contrast, aerosol refers to combined particles and gas. Some particulates occur naturally, originating from volcanoes, dust storms, forest and grassland fires, living vegetation, and sea spray. Human activities, such as the burning of fossil fuels in vehicles, power plants and various industrial processes also generate significant amounts of aerosols. Averaged worldwide, anthropogenic aerosols—those made by human activities—currently account for approximately 10% of our atmosphere. Increased levels of fine particles in the air are linked to health hazards such as heart disease, altered lung function and lung cancer. Particulates are related to respiratory infections and can be particularly harmful to those already suffering from conditions like asthma.

06. Persistent free radicals connected to airborne fine particles are linked to cardiopulmonary disease

07. Toxic metals, such as lead and mercury, especially their compounds.

08. Chlorofluorocarbons (CFCs) – harmful to the ozone layer; emitted from products are currently banned from use. These are gases which are released from air conditioners, refrigerators, aerosol sprays, etc. On release into the air, CFCs rise to the stratosphere. Here they come in contact with other gases and damage the ozone layer. This allows harmful ultraviolet rays to reach the earth's surface. This can lead to skin cancer, eye disease and can even cause damage to plants.

09. Ammonia – emitted mainly by agricultural waste. Ammonia is a compound with the formula NH_3. It is normally encountered as a gas with a characteristic pungent odor. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to foodstuffs and fertilizers. Ammonia, either directly or indirectly, is also a building block for the synthesis of many pharmaceuticals. Although in wide use, ammonia is both caustic and hazardous. In the atmosphere, ammonia reacts with oxides of nitrogen and sulfur to form secondary particles.

10. Odors — such as from garbage, sewage, and industrial processes

11. Radioactive pollutants – produced by nuclear explosions, nuclear events, war explosives, and natural processes such as the radioactive decay of radon.

Secondary pollutants include:

01. Particulates created from gaseous primary pollutants and compounds in photochemical smog. Smog is a kind of air pollution. Classic smog results from large amounts of coal burning in an area caused by a mixture of smoke and sulfur dioxide. Modern smog does not usually come from coal but from vehicular and industrial emissions that are acted on in the atmosphere by ultraviolet light from the sun to form secondary pollutants that also combine with the primary emissions to form photochemical smog.

02. Ground level ozone (O_3) formed from NO_x and VOCs. Ozone (O_3) is a key constituent of the troposphere. It is also an important constituent of certain regions of the stratosphere commonly known as the Ozone layer. Photochemical and chemical reactions involving it drive many of the chemical processes that occur in the atmosphere by day and by night. At abnormally high concentrations brought about by human activities (largely the combustion of fossil fuel), it is a pollutant and a constituent of smog.

03. Peroxyacetyl nitrate ($C_2H_3NO_5$) – similarly formed from NO_x and VOCs.

Minor air pollutants include:

01. A large number of minor hazardous air pollutants. Some of these are regulated in USA under the Clean Air Act and in Europe under the Air Framework Directive

02. A variety of persistent organic pollutants, which can attach to particulates

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Because of this, they have been observed to persist in the environment, to be capable of long-range transport, bioaccumulate in human and animal tissue, biomagnify in food chains, and to have potentially significant impacts on human health and the environment.

There are also sources from processes other than combustion

01. Fumes from paint, hair spray, varnish, aerosol sprays and other solvents. These can be substantial; emissions from these sources was estimated to account for almost half of pollution from volatile organic compounds in the Los Angeles basin in the 2010s.

02. Waste deposition in landfills, which generate methane. Methane is highly flammable and may form explosive mixtures with air. Methane is also an asphyxiant and may displace oxygen in an enclosed space. Asphyxia or suffocation may result if the oxygen concentration is reduced to below 19.5% by displacement.

03. Military resources, such as nuclear weapons, toxic gases, germ warfare and rocketry.

04. Fertilized farmland may be a major source of nitrogen oxides

Natural sources

01. Dust from natural sources, usually large areas of land with little vegetation or no vegetation

02. Methane, emitted by the digestion of food by animals, for example cattle

03. Radon gas from radioactive decay within the Earth's crust. Radon is a colorless, odorless, naturally occurring, radioactive noble gas that is formed from the decay of radium. It is considered to be a health hazard. Radon gas from natural sources can accumulate in buildings, especially in confined areas such as the basement and it is the second most frequent cause of lung cancer, after cigarette smoking.

04. Smoke and carbon monoxide from wildfires. During periods of actives wildfires, smoke from uncontrolled biomass combustion can make up almost 75% of all air pollution by concentration.

05. Vegetation, in some regions, emits environmentally significant amounts of Volatile organic compounds (VOCs) on warmer days. These VOCs react with primary anthropogenic pollutants—specifically, NO_x, SO_2, and anthropogenic organic carbon compounds — to produce a seasonal haze of secondary pollutants.[33] Black gum, poplar, oak and willow are some examples of vegetation that can produce abundant VOCs. The VOC production from these species result in ozone levels up to eight times higher than the low-impact tree species.

06. Volcanic activity, which produces sulfur, chlorine, and ash particulates

Emission factors

Air pollutant emission factors are reported representative values that attempt to relate the quantity of a pollutant released to the ambient air with an activity associated with the release of that pollutant. These factors are usually expressed as the weight of pollutant divided by a unit weight, volume, distance, or duration of the activity emitting the pollutant (e.g., kilograms of particulate emitted per tonne of coal burned). Such factors facilitate estimation of emissions from various sources of air pollution. In most cases, these factors are simply averages of all available data of acceptable quality, and are generally assumed to be representative of long-term averages.

There are 12 compounds in the list of persistent organic pollutants. Dioxins and furans are two of them and intentionally created by combustion of organics, like open burning of plastics. These compounds are also endocrine disruptors and can mutate the human genes.

The United States Environmental Protection Agency has published a compilation of air pollutant emission factors for a wide range of industrial sources.[35] The United Kingdom, Australia, Canada and many other countries have published similar compilations, as well as the European Environment Agency.[36][37][38][39]

Air pollution risk is a function of the hazard of the pollutant and the exposure to that pollutant. Air pollution exposure can be expressed for an individual, for certain groups (e.g. neighborhoods or children living in a country), or for entire populations. For example, one may want to calculate the exposure to a hazardous air pollutant for a geographic area, which includes the various microenvironments and age groups. This can be calculated[2] as an inhalation exposure. This would account for daily exposure in various settings (e.g. different indoor micro-environments and outdoor locations). The exposure needs to include different age and other demographic groups, especially

infants, children, pregnant women and other sensitive subpopulations. The exposure to an air pollutant must integrate the concentrations of the air pollutant with respect to the time spent in each setting and the respective inhalation rates for each subgroup for each specific time that the subgroup is in the setting and engaged in particular activities (playing, cooking, reading, working, spending time in traffic, etc.). For example, a small child's inhalation rate will be less than that of an adult. A child engaged in vigorous exercise will have a higher respiration rate than the same child in a sedentary activity. The daily exposure, then, needs to reflect the time spent in each micro-environmental setting and the type of activities in these settings. The air pollutant concentration in each microactivity/microenvironmental setting is summed to indicate the exposure.[2] For some pollutants such as black carbon, traffic related exposures may dominate total exposure despite short exposure times since high concentrations coincide with proximity to major roads or participation to (motorized) traffic. A large portion of total daily exposure occurs as short peaks of high concentrations, but it remains unclear how to define peaks and determine their frequency and health impact

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1. An Introduction to Air Sampling

Health and Safety in the workplace involves a wide range of potential hazards:

Airborne hazards from gases, vapours, dusts and fibres can all cause potentially life threatening illnesses affecting the lungs, kidneys and liver. Air sampling is a vital method of monitoring workers' exposure to these potential airborne workplace hazards. Air sampling is relevant to almost every industry, from flour dust exposure in a bakery to chemical vapour exposure in a factory. Every year in the UK, around 13,000 people die from diseases which were caused by the work that they do, or used to do. By comparison, in 2017/2018 only 144 workers were killed at work due to fatal injuries (source: HSE). By consistently measuring the concentration of airborne contaminants steps can be taken to reduce workers' exposure, thereby helping to prevent chronic respiratory diseases such as asthma, as well as occupational cancers.

What is Air Sampling?

Air sampling is carried out to ensure that workplace or environmental air is meeting regulatory standards and to help Occupational Hygiene and Health & Safety professionals assess employee exposure to airborne hazards. Regulatory authorities, such as the HSE in the UK or OSHA in the USA, apply limits for exposure to most substances deemed hazardous to worker's health. The HSE terms these limits Workplace Exposure Limits (WELs) and they are defined as the maximum concentration of a hazardous airborne substance that a worker may be exposed to over a defined period such as an 8-hour shift. WELs apply to anybody working within an environment where hazardous substances are present, and are based on personal sampling, not static / environmental – however, this does not mean that limits cannot apply under these circumstances.

Types of contaminant:

Control of Sustances Hazardous to Health Regulations 2002 (COSHH) covers substances that are hazardous to health.

Most hazardous materials fall into three main categories: dusts and particulates, gases and vapours, or bioaerosols. A site may well have several types of contaminants.

1. Dusts:
    1. Present in almost every industry
    2. Risks to health from inhalation exposure, but also other hazards such as risk of explosion
    3. Examples include dusts from wood, flour, metals and silica

2. Fibres:
    1. Longer than they are wide
    2. Can be highly toxic due to size, shape and a tendency to remain in the lungs
    3. Examples include asbestos, ceramic fibres, or mineral fibres

3. Gases and Vapours:
    1. Vast range of potentially harmful and explosive gases and vapours across industries
    2. Can be harmful to the body due to toxicity leading to acute or chronic conditions
    3. Presence of non-toxic gases can also be dangerous due to displacement of oxygen
    4. Can also be harmful through skin absorption

4. Bioaerosols:
    1. Can be present in many environments, including waste management, recycling facilities and composting sites
    2. Could be infectious, produce toxins, or trigger an immune response
    3. Examples include airborne viruses, fungal spores, bacteria and pollen

Measurements

Once measured, the contaminants are expressed as milligrams per cubic metre (mg/m^3) for particulates and parts per million (ppm) for gases. For instance, the maximum workplace exposure limit for total (inhalable) dust is 10 mg/m^3 over an 8h period. 1 milligram per cubic metre (mg/m^3) is approximately the same as one teaspoon of dust spread over the area of a football field to a height of one metre. 1 part per million (ppm) is approximately the same as the contents of a party balloon compared to the volume of air inside 50 three bedroom houses

Air Samplers for Microbiological Monitoring of Air Quality

Key Points

01. Active air sampling delivers a quantitative result
02. Modern samplers are convenient and easy to use
03. Operation can be semi-automated
04. Potential for integration with environmental monitoring software

Introduction to Microbiological Air Sampling

Ask a microbiologist to list the most important sources of contamination in any specific manufacturing operation and they will probably come up with people, raw materials and water as the top three. This is a perfectly reasonable response, but there is one other factor that is always present and that is air. Viable microorganisms can be found in the atmosphere almost anywhere – bacterial spores can be isolated from the jet stream several miles above the Earth's surface – but the microbiology of the air is sometimes overlooked.

Airborne bacterial and fungal cells and spores may be present in droplets as bioaerosols, as very small individual particles that stay suspended for long periods, or as larger clumps and aggregates that settle rapidly onto surfaces. They can be an important source of infection in medical facilities and can contaminate sensitive manufacturing operations, but regular monitoring of airborne microorganisms is sometimes neglected.

Microbiological monitoring of the air in facilities where pharmaceuticals and medical devices are produced is essential and well established. In most countries it is a regulatory requirement, and international standards have been published for biocontamination control in cleanrooms and other controlled environments (ISO 14698-1/2). But airborne bacteria and fungi may be equally important in hospitals, in food factories and even in office buildings and other working environments. For example, high levels of airborne fungal spores in bakeries may have a significant negative effect on product shelf life, and airborne microorganisms may also be a contributory factor in episodes of so-called 'sick building syndrome'. Monitoring airborne microorganisms is therefore a key component of environmental monitoring in many sectors and a range of technological solutions has been developed to help operators achieve an effective monitoring programme, not just in the pharmaceutical sector, but in hospitals, food factories and a variety of other environments.

Technology

There are two principal means of monitoring the microbiological population of the air, passive monitoring and active sampling. Both have a part to play, but active sampling methods have become an essential environmental monitoring tool, especially in the pharmaceutical and medical device sectors.

Passive monitoring

Passive monitoring is usually done using 'settle plates' – standard Petri dishes containing appropriate (usually non-selective) culture media that are opened and exposed for a given time and then incubated to allow visible colonies to develop and be counted. Settle plates are very limited in their application since they are only really capable of monitoring viable biological particles that sediment out of the air and settle onto a surface over the time of exposure. They will not detect smaller particles or droplets suspended in the air and they cannot sample specific volumes of air, so the results are not quantitative. They are also vulnerable to interference and contamination from non-airborne sources and the agar growth medium in the plates may deteriorate if they are exposed for too long. Settle plates may easily become overgrown in heavily contaminated conditions and interpretation of the data they produce can be difficult.

On the other hand, settle plates are inexpensive and easy use, requiring no special equipment. They are useful for qualitative analysis of airborne microorganisms and the data they produce may detect underlying trends in airborne contamination and provide early warning of problems. They are also useful for directly monitoring airborne contamination of specific surfaces. In an environment such as a low risk food factory, settle plates may provide an adequate means of monitoring biological air quality.

Active monitoring

Active monitoring requires the use of a microbiological air sampler to physically draw a known volume of air over, or through, a particle collection device and there are two main types.

01. **Impingers:** Impingers use a liquid medium for particle collection. Typically, sampled air is drawn by a suction pump through a narrow inlet tube into a small flask containing the collection medium. This accelerates the air towards the surface of the collection medium and the flow rate

is determined by the diameter of the inlet tube. When the air hits the surface of the liquid, it changes direction abruptly and any suspended particles are impinged into the collection liquid. Once the sampling is complete the collection liquid can be cultured to enumerate viable microorganisms. Since the sample volume can be calculated using the flow rate and sampling time, the result is quantitative. Impingers have disadvantages for routine microbiological monitoring of the air. Traditional designs are usually made of glass, which is undesirable in food and pharmaceutical production sites. Impingement into liquids may also damage some microbial cells and affect viability and overlong sampling times may allow some cells to multiply in the liquid collection medium. However, the liquid collection medium means that the sample can be analysed using a variety of methods, including molecular techniques such as PCR, so that results can be obtained more rapidly. Instruments have been developed using variations on the impinger design, such as the Coriolis®μ sampler made by Bertin Technologies, and the SAS-PCR sampler from VWR-pbi, which are not constructed from glass and can be used to sample the air in clean rooms and other controlled environments. The Coriolis sampler uses a cyclone effect to accelerate the sampled air into the collection liquid. Any suspended particles in the air are thrown out by centrifugal force, collect on the walls of the conical collection vessel and concentrate in the collection liquid. The SAS-PCR device is designed specifically to collect pathogens for subsequent detection by molecular methods and circulates the collection liquid to prolong contact time with sampled air.

02. **Impactors:** Impactor samplers use a solid or adhesive medium, such as agar, for particle collection and are much more commonly used in commercial applications than impingers, largely because of their convenience. In a typical impactor sampler air is drawn into a sampling head by a pump or fan and accelerated, usually through a perforated plate (sieve samplers), or through a narrow slit (slit samplers). This produces laminar air flow onto the collection surface, often a standard agar plate or contact plate filled with a suitable agar medium. The velocity of the air is determined by the diameter of the holes in sieve samplers and the width of the slit in slit samplers. When the air hits the collection surface it makes a tangential change of direction and any suspended particles are thrown out by inertia, impacting onto the collection surface. When the correct volume of air has been passed through the sampling head, the agar plate can be removed and incubated directly without further treatment. After incubation, counting the number of visible colonies gives a direct quantitative estimate of the number of colony forming units in the sampled air. Impaction samplers offer benefits in terms of convenience and pre-poured, gamma-irradiated contact plates and standard petri dishes from specialist suppliers can

be used with them to minimise the risk of contamination and variation. They are also able to handle higher flow rates and the large sample volumes necessary to monitor air quality in clean rooms where the number of microbes present is likely to be very low. However, care must be taken not to allow agar plates to remain in the sampler heads for too long, or the medium may dry out and deteriorate. Microbial cells may also be damaged by mechanical stress during the sampling process and lose viability. Most impaction samplers also do not allow the use of rapid methods to enumerate and characterise microorganisms, but rely on conventional culture for several days to obtain a result. This problem can be overcome to some extent by the use of a water-soluble polymer gel instead of agar. This allows the sample to be analysed by rapid techniques such as PCR or cytometry. A wide variety of instruments have been developed using the impaction principle. One of the best known is the Andersen sampler, a multi-stage 'cascade' sieve sampler that uses perforated plates with progressively smaller holes at each stage, allowing particles to be separated according to size. Another well known instrument is the Casella slit sampler, in which the slit is positioned above a turntable on which is placed an agar plate. As air is drawn through the slit, the agar plate rotates, so that particles are deposited evenly over its surface. Both of these instruments have been used for many years, but more recently a number of highly portable and convenient impaction samplers have been developed specifically for monitoring the air in production facilities and other sensitive areas. Most of these are sieve samplers, such as the Surface Air System (SAS) samplers made by VWR-pbi in Italy, and use agar contact plates or full-sized culture plates as the collection surface. However, some types, such as the RCS samplers from Merck Millipore, use a centrifugal impeller to accelerate air onto a dedicated agar-coated strip that can be incubated directly. These portable samplers can be hand-held, or mounted on a tripod during sampling, and can be programmed to sample a specific volume of air, or sequential samples at pre-set times. Samplers specifically designed to monitor the microbiological quality of compressed gases are also available. Semi-automated systems, usually based on sieve type impaction samplers, are also available for monitoring clean rooms and controlled production areas. These systems typically use a number of sampler heads linked to a central control unit, which can be programmed to follow a pre-set sampling programme. The sampler heads can be fitted permanently in place so that they undergo the same sterilisation regime as the rest of the clean room. It is also possible to set up a wireless network of portable air samplers controlled by a central PC, with no need for any electrical or vacuum line connections. Semi-automated systems often allow integration with environmental monitoring and QC software packages, such as MODA-EM from Lonza, providing the basis of a paperless system for recording microbiological data.

Other types

Most commercially available microbiological air samplers use the impaction or impingement collection techniques, but other types of sampler are also used in some applications. The most commonly used alternative is filtration, where the air is drawn by a pump or vacuum line through a membrane filter. The filter medium may be polycarbonate or cellulose acetate, which can be incubated directly by transferring onto the surface of an agar medium, or gelatine, which can be dissolved and analysed by culture or rapid methods. Filtration methods are accurate and reliable and portable filtration samplers designed for the pharmaceutical industry are available. However, filtration is less convenient than impaction-based sampling and may cause dehydration stress in the trapped microorganisms.

Recently, instruments have been developed that are capable of detecting airborne microorganisms in real time. These employ laser technology to induce fluorescence in any viable particles in air drawn through the instrument and provide immediate detection and enumeration of microbial contaminants. An example is the BioLaz® instrument from Particle Measuring Systems, which is designed specifically for use in the pharmaceutical and medical products sectors. TSI's BioTrak® Real-Time Viable Particle Counter instrument uses similar Laser Induced Fluorescence (LSI) technology, but is also capable of simultaneous total and viable particle counts.

Air Sampler Validation and Calibration

Microbiological air sampling in clean rooms and other controlled environments usually requires the sampling of large volumes of air (at least $1m^3$). It is also very important that samples are representative and the results of sampling accurate enough to ensure that the air meets regulatory standards, or guidelines. It is therefore essential that air samplers are properly validated and regularly calibrated to ensure accuracy. There are a number of points to consider.

01. Physical efficiency of the sampler – the relative efficiency of the sampler in collecting particles over a range of sizes.

02. Biological efficiency – the relative efficiency of the sampler in collection of microorganisms on a surface or in a liquid so that they are viable and can be counted.

03. Validation of the instrument for its intended application and environment.

04. The flow rate of the sampler – with large sample sizes, the flow rate of air through the sampling head is critical to the accuracy of the result.

Methods for estimating physical and biological efficiency are given in the ISO 14698-1 standard. Sampler manufacturers typically employ a third party laboratory to validate their instruments in this way, by challenging the instrument with particles and microorganisms in a controlled chamber. Physical efficiency is normally measured against membrane filtration sampling, while biological efficiency is compared with an established reference sampler such as the Casella slit sampler.

Validation for specific applications often requires operating a new sampling method in parallel with an existing system for a period sufficient to provide a valid comparison. Many sampler suppliers provide validation methods and recommendations for their instruments.

The flow rate of the sampler pump or fan should be properly calibrated and validated against a certified flow meter, preferably by a third party, at least once every twelve months and should also be regularly calibrated by the user to ensure that the sampler has not developed a fault or suffered any damage. Some sampler manufacturers offer a validation service and equipment, typically an anemometer, to calibrate samplers on site.

II. Aerosols

An aerosol is defined as a suspension system of solid or liquid particles in a gas. An aerosol includes both the particles and the suspending gas, which is usually air.[1] Frederick G. Donnan presumably first used the term aerosol during World War I to describe an aero-solution, clouds of microscopic particles in air. This term developed analogously to the term hydrosol, a colloid system with water as the dispersed medium.[4] Primary aerosols contain particles introduced directly into the gas; secondary aerosols form through gas-to-particle conversion.

Various types of aerosol, classified according to physical form and how they were generated, include dust, fume, mist, smoke and fog.

There are several measures of aerosol concentration. Environmental science and environmental health often use the mass concentration (M), defined as the mass of particulate matter per unit volume, in units such as $\mu g/m^3$. Also commonly used is the number concentration (N), the number of particles per unit volume, in units such as number per m^3 or number per cm

Particle size has a major influence on particle properties, and the aerosol particle radius or diameter (d_p) is a key property used to characterise aerosols.

Aerosols vary in their dispersity. A monodisperse aerosol, producible in the laboratory, contains particles of uniform size. Most aerosols, however, as polydisperse colloidal systems, exhibit a range of particle sizes. Liquid droplets are almost always nearly spherical, but scientists use an equivalent diameter to characterize the properties of various shapes of solid particles, some very irregular. The equivalent diameter is the diameter of a spherical particle with the same value of some physical property as the irregular particle. The equivalent volume diameter (d_e) is defined as the diameter of a sphere of the same volume as that of the irregular particle. Also commonly used is the aerodynamic diameter.

III. Medium for air microbial quality testing

A wide selection of ready-to-use agar media and testing solutions is available from Merck for reliable active air monitoring to keep your manufacturing facilities – and thus the products you produce – safe. These high quality settle plates, contact plates, agar strips and media cassettes are ideally suited for use with our proven active air monitoring systems – and they are available in product variants specifically for use in either less critical or highly critical clean rooms and isolators. In combination, they match perfectly and conveniently for the monitoring of ambient air and compressed gases – for rapid and reliable results in the course of your environmental monitoring routines.

Our products are developed in close cooperation with users and authorities – to make sure they are perfectly tailored to the needs of our customers and they follow international guidelines for critical and controlled environments such as EU, cGMP, USP or FDA Aseptic Guidance.

Contact Plates

Contact plates are the perfect match for our MAS-100® air sampling systems. They are available for non-critical areas as well as for critical areas.

01. HYCON® Agar Strips: HYCON® agar strips are the perfect media for use with the RCS® microbial air monitoring systems. These strips are available as total count or selective media for specific microorganisms.
02. M Air T® Cassettes: M Air T® cassettes are the perfect media for use with the M Air T® microbial air samplers. Our pre-filled agar cassettes are sterile and easy to load.

Settle Plates

Settle plates are the ideal media for use with the MAS-100® microbial air sampling systems. They are available as gamma-irradiated and non-irradiated settle plates.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – III – Air & Water Microbiology – SMB2203

The bio-air sterilization system removes harmful contaminants and particulates, such as bacteria, viruses and molds, from air within an enclosed area, as well as from the surfaces located within the enclosed area, while also maintaining a safe workplace for individuals. The bio-air sterilization system uses a compressor to circulate indoor air through a pre-ionization field, an air filter, a dual ultraviolet system located within the bio-air sterilization system.

People spend 75-90% of their time indoors where they are exposed to a growing number of health-threatening indoor pollutants. These pollutants can be categorized into three groups: biological contaminants, such as bacteria, viruses and molds; toxic gases and fumes given off by furniture, carpeting, etc.; and particulates, such as dust and smoke. Approximately half of the major office buildings have contaminated heating, ventilation and air conditioning (HVAC) systems. If not properly maintained, the HVAC systems are a hotbed for the growth of molds and bacteria, regardless of the age of the building. Occupants of these buildings can be expected to suffer from symptoms related to exposure to these health-threatening indoor pollutants. The problem of health-threatening indoor pollutants is exacerbated when the building is a health facility where not only are there a greater number of harmful health-threatening pollutants present, but occupants of the health facility may be more susceptible to maladies caused by these health-threatening pollutants.

A known solution for removing harmful contaminants from ambient air is the use of air purifiers. Air purifiers use a scientifically advanced process that combines the power of genocidal ultraviolet (UV) light, purifying hydroxyl, activated oxygen and photo-ionization for purifying air and sanitizing an area. However, existing air purifiers don't use the multiple approach of pre-ionization of the incoming air, HEPA filtration, and sterilization by use of ultraviolet nm lamps for a more complete solution. Further, most existing air purifiers use small ultraviolet lamps that do not allow adequate time required for sterilization.

Another known solution for removing harmful contaminants from surfaces is the use of chlorine to clean water and surfaces. However, chlorine may leave harmful residuals within the drinking water and chlorine also cannot be readily generated on site. The chlorine must be shipped to the site from manufacturers located a distance away from the point of need. During emergency situations, proper handling of chlorine containers may be impractical.

Because neither of the above-stated solutions is adequate, there is a need for a sterilization system that effectively filters out particulates, safely generates ozone for destroying biological contaminants, and effectively converts the ozone to highly ionized ambient air.

The above-described problems are solved by the present bio-air sterilization system that removes harmful contaminants and particulates, such as bacteria, viruses and molds, from air within an enclosed area, as well as from the surfaces located within the enclosed area, while also maintaining a safe workplace for individuals. The bio-air sterilization system uses a compressor to circulate indoor air through an electrostatic filter that generates an pre-ionization field, a mechanical air filter, a dual ultraviolet system located within the bio-air sterilization system.

The pre-ionization field is created by the electrostatic filter when a voltage is applied between the outside shell and the wire mesh of the air filter to pre-ionize the incoming indoor air. The power supply (transformer) applying the charge is fluttered at a high rate (1000 P/s) to create an ionization field for the incoming indoor air. Ions produced by the pre-ionization field attach to particulates in the incoming air, giving them a negative or positive charge so that the particulates may attach to the air filter as the compressor/blower circulates indoor air through the bio-air sterilization system

The ultraviolet light system consists of two ultraviolet lamps having different radiation wavelengths. The first ultraviolet lamp, operating at 254.2 nanometers (nm) destroys airborne contaminants and particulates in the enclosed area while in operation for a pre-determined period of time. The ions generated by the first ultraviolet lamp attach to airborne particulates and contaminants, giving them a negative or positive charge so that the particles and contaminants attach themselves to nearby surfaces and thereby settle out of the air. At a pre-set time, the first ultraviolet lamp deactivates and the second ultraviolet lamp, operating at 185 nm, is activated and converts oxygen into ozone which is circulated throughout the enclosed area to destroy harmful surface contaminants and particulates. The enclosed area is not safe for occupancy during the time the second ultraviolet lamp is operational at 185 nm. An integrated timer in the programmable logic controller allows for customized control of the second ultraviolet lamp's period of operation. As an additional safety feature, the bio-air sterilization system is fitted with a motion sensor that deactivates the second ultraviolet lamp if someone should enter the enclosed are Again at a pre-set time, the second ultraviolet lamp is deactivated and the first ultraviolet lamp is reactivated, operating at 254.2 nm for an appropriate period of time to kill newly introduced airborne contaminants and excess ozone generated by the 185 nm lamp, making the area again safe for occupancy.An optional, commercial pre-filter can be used for enclosed areas having an excessive amount of dust and/or other heavy particulates in the air.

Alternatively, a corona discharge ozone generator can be substituted for the second ultraviolet lamp to generate higher concentrations of ozone for applications, such as a medical facility, that require higher levels of contaminant destruction.

The use of a dual internal ultraviolet system is a photocatalytic oxidation process that combines ultraviolet light of the two different wavelengths, oxygen and the natural humidity found in air, enhanced by the use of high frequency ballast 126 to activate the ultraviolet lamps to create what is known as a hydroxyl radical. The hydroxyl radical is an extremely aggressive oxidizer that neutralizes airborne contaminants and biological and chemical odors within milliseconds, converting them to harmless carbon dioxide and water vapor.

Air Purification/Sterilization Using Ultraviolet/Ozone

The human population is continually exposed to a growing number of health-threatening pollutants that can be categorized into three groups: biological contaminants, toxic gases, and particulates. To deal with these hazards, the present bio-air sterilization system utilizes pre-ionization, filtration, ultraviolet radiation and ozone generation for air purification. First, a voltage supplied by the transformer 128 is applied between the outer perforated stainless steel shell of the pre-ionization field 100 and the internal wire mesh of the filter 112 to pre-ionize the incoming air. Second, the air passes through the high efficiency particulate air (HEPA) filter 112 to which particulates are attached. Third, special wavelengths (254.2 nm) of concentrated ultraviolet light are used to destroy proteins and DNA and in the process, kill harmful airborne contaminants such as bacteria, viruses and molds that depend on these biochemicals for life function. Fourth, photonics ozonation (ultraviolet light at a wavelength of 185 nm) is used to eliminate unpleasant odors, neutralize many harmful gases, and destroy surface contaminants such as bacteria, viruses and fungi. Fifth, purifying hydroxyl, one of the strongest pollutant fighters of all, further destroys surface contaminants. Finally, ions are produced within the dual lamp region and attach themselves to airborne particulates such as dust, pollen, soot, smoke, etc., removing them from the indoor air.

The present bio-air sterilization system is designed for installation in any enclosed area requiring the destruction of bacteria, viruses and odors. For extremely large areas, two or more bio-air sterilization systems may be required and can operate concurrently. The system utilizes a scientifically advanced process that combines the power of genocidal ultraviolet light, purifying hydroxyl, activated oxygen and photo-ionization for the bio-air sterilization system to purify and sanitize indoor air in a defined area. As air passes the radiant field of the special 185 nm ultraviolet lamp 124 it is exposed to a powerful purifying process that destroys harmful contaminants to disinfect surfaces and recessed areas where pathogens may collect. The ultra-purified air is then re-circulated back into the environment and through the radiant field of the first ultraviolet lamp 122 which destroys airborne

contaminants which have been newly introduced into the enclosed area and, more importantly, destroys excess ozone produced by the second ultraviolet lamp 124, making the area safe for inhabitants. The air has then become highly ionized, carrying negative ions for removal of particulates from the air and low, safe levels of photonics ozone to seek out and destroy additional hazardous contaminants.

The dual internal ultraviolet system includes operation of a first ultraviolet lamp 122, which operates at a predetermined wavelength of, for example, 254.2 nm, to destroy biological contaminants such as airborne viruses, bacteria, molds, fungi, smoke, and pollution particles. Subsequently, when the second ultraviolet lamp 124, which operates at a predetermined wavelength of, for example, 185 nm, is activated, not only are surface biological contaminants destroyed in the ultraviolet field, but simple oxygen from the indoor air is converted to one of the strongest oxidizers on the planet, ozone. This photocatalytic oxidation process combines ultraviolet light of two different wavelengths, oxygen and the natural humidity found in air, enhanced with a high frequency ballast 126 to create what is known as a hydroxyl radical. The ballast power source for lamp operation 126 utilizes constant wattage circuitry to ensure optimum electronic efficiency and consistent performance. As noted above and as described in more detail below, a corona discharge ozone generator can be substituted for the second ultraviolet lamp to perform the ozone generation function.

Referring to FIG. 3, the cycle of the present bio-air sterilization includes six stages. In Stage 1, the indoor air is pre-ionized. Ionization through a wire mesh field 100 prior to filtration produces ions that attach to biological contaminants, making the biological contaminants heavier and more likely to be filtered out, as, at Stage 2, the ionized air passes through the HEPA filter 112.

During Stage 3, the filtered air passes through the radiant field of a first ultraviolet lamp 122 where many of the airborne biological contaminants, passing through the radiant field of ultraviolet light at 254.2 nm generated by the first ultraviolet lamp 122, are destroyed. During Stage 4, the ultraviolet light emissions at a wavelength of 185 nm, generated by the second ultraviolet lamp 124, convert the filtered air to ozone as the indoor air circulates through the bio-air sterilization system. Replacing the indoor air with ozone for a period of time destroys surface biological contaminants in addition to any airborne contaminants within the room. At Stage 5, residual ozone and any newly introduced airborne contaminants are destroyed when the first ultraviolet lamp 122 is re-activated and a high level of negative ionization is achieved. At Stage 6 the compressor/blower 130 recirculates the highly ionized air through exit tube 132 and into a mixing chamber 134 (an empty stainless steel box where ozone is mixed by creating turbulence) prior to re-entry into the enclosed area through a directional output

nozzle 136 at the top of the unit. The continuous cycle through the bio-air sterilization system then begins again.

Stage One: Pre-ionization

The present bio-air sterilization system contains a built-in pre-ionization field 100. The outside shell of the pre-ionization field 100 is constructed of perforated stainless steel while the outside shell of the interior filter system 112 is covered with a stainless steel wire mesh. A low voltage, 1000 Hz power supply connected to the outside shell applies a + (positive) charge to the outside shell while the same power supply applies a − (negative) charge to the interior stainless steel mesh. This produces an electrical field that charges the airborne contaminants to promote aggregation of particles for easy removal and actually electrocutes a large portion of the airborne pathogens. The power supply is a low voltage, low amperage dc system located within the bio-air sterilization system. The power supply output is then fluttered at over 1,000 times per second to create an ionizing field for the entering indoor air to produce ions.

Stage Two: Filtration

The ions produced in stage one attach to particles in the air giving them a negative or positive charge so that the particles may attach to nearby surfaces within the air filter 112 during the air filtration stage. The air filter 112 meets HEPA specifications to capture particle sizes as small as 0.3 to 0.5 microns. (The smallest particle size visible to the human eye is 40 microns.)

The HEPA filter 112 may be pleated to ensure proper seal and to maximize surface area. The pleated design yields a surface area several thousand times that of a flat filter using the same space requirements. The filter 112 traps a significant amount of the airborne contaminants and particulates such as pollen, molds, and dust that are circulating in the room.

Stage Three: Radiation

The present bio-air sterilization system includes a dual internal ultraviolet system. The daytime operation utilizes a first ultraviolet lamp 122, operating at a wavelength of 254.2 nm, and the nighttime operation utilizes a second ultraviolet lamp 124, operating at a wavelength of 185 nm. Use of a dual internal ultraviolet lamp system is a photocatalytic oxidation process that combines ultraviolet light of two different wavelengths, oxygen, and the natural humidity found in air, enhanced by the use of a high frequency ballast 126 to activate the ultraviolet light generators to create what is known as a hydroxyl radical. The hydroxyl radical is an extremely aggressive oxidizer that neutralizes airborne contaminants and biological and chemical odors within milliseconds, converting them to harmless carbon dioxide and water vapor. Following the first and second stages illustrated in FIG. 3, the third stage exposes the filtered air from the HEPA filter to a radiant field created by a first ultraviolet lamp 122 operated at a wavelength of 254.2 nm to destroy airborne contaminants. This lamp is usually operated during the day when the enclosed area is occupied and disinfection must be restricted to the air passing through the filter system.

Stage Four: Ozone Shock Treatment

Prior to operation of the second ultraviolet lamp 124, the room is vacated. Since most enclosed areas are, presumably, occupied during the daytime, it is assumed the optimum operational hours for the second ultraviolet lamp 124 are at nighttime when most contaminated areas are unoccupied. However, the second ultraviolet lamp 124 can be preset through the programmable logic controller 140 to be operational during any specified period. Although the bio-air sterilization system produces a low concentration of ozone, prolonged exposure could be harmful to human beings. Therefore, the enclosed area should be vacant while ozone is being produced. As an additional precaution, a motion sensor 138 deactivates the second ultraviolet lamp 124 if someone should enter the room while the second ultraviolet lamp 124 is operational. As the filtered air circulates through the radiant field created by operation of the second ultraviolet lamp 124, harmful surface contaminants such as bacteria and viruses are destroyed. The ultraviolet lamps are fired by a ballast 126 having a cycle rate of 35,000 cycles per second compared to the standard cycle rate of 24,000 cycles per second as used on conventional ultraviolet systems. This higher cycle rate greatly enhances the output of the lamp in microwatts per square centimeter.

Operation of the second ultraviolet lamp 124 also converts oxygen from the filtered air into one of the strongest oxidizers on the planet, ozone. The ozone then leaves the bio-air sterilization system

and is distributed throughout out the enclosed area. When bacteria and viruses come in contact with the ozone the destruction is immediate. Since the ozone is airborne, it can reach all of the places bacteria and viruses reach, such as bedding, carpet, furniture, floors, walls, the inside of walls, etc. Depending on the contamination in the area to be sterilized and the size of the area, the duration of operation of the second ultraviolet lamp 124 may vary, but it is timed through the programmable logic controller 140 to deactivate well before the area is scheduled for occupancy again.

The optional corona discharge ozone generator makes use of multiple anode/cathode cells. Ozone is created when a gas (ambient air or oxygen) passes through the space between the anode and cathode while a high voltage is applied across the anode and cathode arrangement. As the gas passes through the space between the anode and cathode, the electric field created by the voltage applied to the anode and cathode splits some oxygen molecules, and the released oxygen atoms react with oxygen molecules to form ozone. The corona discharge pack is constructed of a rolled stainless steel, perforated shell. A ceramic dielectric is inserted into the shell and a working gap is established between the ceramic dielectric and the shell to support the corona discharge. The commercial low voltage power is stepped-up to the high voltage necessary to create the corona by a frequency/voltage inverter before delivery to the ceramic core. As it passes through the corona, the oxygen in ambient air becomes excited and its oxygen molecules become disassociated. Some of the oxygen molecules recombine into ozone. The corona discharge ozone generator is designed to produce ozone levels adequate for the required room disinfection. For simplicity of description, the following description refers to a “second ultraviolet lamp” and it is understood that the corona discharge ozone generator can be substituted for the second ultraviolet lamMore and more room furnishings and floor coverings are made from synthetic materials. Under the stagnant air conditions that prevail due to the lack of ventilation, decomposition products and solvents leach out of these synthetic materials and fill the indoor space. Much has been said about the “sick building syndrome” which usually refers to institutional buildings—but private homes can have basically the same problems. When odors are inhaled, they cause an increase in the demand for oxygen in the human body, thus depleting the body of oxygen. Ozone reacts rapidly with most odors, oxidizing them into less harmful elements or into harmless carbon dioxide.

Ozonization

Ozone in natural settings is around 0.02 parts per million (ppm), but it can be as high as 0.10 ppm. At this level, ozone is capable of keeping pathogens in check, and yet, at this level, ozone is not harmful to higher life forms such as fish, birds, animals, or man. Ozone is not harmful to nature. Only a prolonged exposure to unnaturally high levels of ozone may lead to discomfort (headache and coughing), signaling one to leave that space and seek better air.

The purpose of the ozone shock treatment is to eliminate contamination from the air and surfaces and provide continuous control over recurring contamination by way of air filtration, ozone, and ultraviolet exposure over a period of time. When ozone comes into contact with bacteria, viruses, fungi and molds, it gives up an atom of oxygen (a free radical) that oxidizes, or destroys, the contaminants. These life forms are anaerobic and cannot live with activated oxygen.

The extent of the odor and contaminant problem determines the size of the ozone generator (bio-air sterilization system) required to sufficiently eliminate the problem of odor, bacteria, and viruses in a reasonable period of time. Once the ideal size of the bio-air sterilization system has been determined, the bio-air sterilization system should be placed in a direction that enhances air circulation in the problem area.

Breathing ozone or drinking ozonated water (at the safe, legal concentrations that are already outlined by the government) are two of the ways to get activated oxygen into a human body. If you have ever taken a drink of water just down stream from a waterfall and felt invigorated, it was because the water had tumbled over the rocks, thinned out and absorbed oxygen/ozone from the air. If it were unsafe to breathe acceptable concentrations of ozone, the military would not use ozone to purify the air in submarines. Oxygen is the first line of defense for the human immune system and is necessary for the removal of every waste product from our bodies.

Stage Five: Ozone Destruction and Ionization

When the second ultraviolet lamp 124 is deactivated and the first ultraviolet lamp 122 is reactivated, the compressor/blower 130 re-circulates the air through the bio-air sterilization system and destroys new airborne contaminants brought into the room and the excess ozone that was generated by the second ultraviolet lamp 124 while the area was unoccupied. The radiant field created by the first ultraviolet lamp 122 not only destroys airborne viruses and bacteria reintroduced by occupants,

recirculation of the air through the bio-air sterilization unit also destroys the excess ozone generated by the second ultraviolet lamp 124.

The first ultraviolet lamp 122 also operates as an ionizer, or ion generator. An ionizer disperses negatively and/or positively charged ions into the air. Ions are charged particles in the air that are formed when enough energy acts upon a molecule such as carbon dioxide, oxygen, water or nitrogen to eject an electron from the molecule leaving a positively charged ion. The displaced electron attaches itself to a nearby molecule that then becomes a negatively charged ion.

These ions attach to particles in the air, giving them a negative or positive charge so that the particles may attach to nearby surfaces such as walls or furniture, or attach to one another and settle out of the air. Human exposure to airborne contaminants is considerable; but ionization of the contaminants removes them from the air rendering the air safe to breathe. Circulation of indoor air through the radiant field created by the first ultraviolet lamp 122 destroys airborne contaminants.

Stage Six: Recirculation

A compressor/blower 130 circulates ambient air through the bio-air sterilization system. The compressor/blower 130 operates at approximately 65 cubic feet per minute (CFM) to allow time for the ambient air, following filtration, to reside in the ultraviolet system for destruction of harmful contaminants.

The purified air recirculates through exit tube 132 that extends from the bottom of the bio-air sterilization cabinet 102 through a mixing chamber 134 (an empty stainless steel box where ozone is mixed by turbulence created by “bouncing” of the walls of the box) and exits out into the enclosed area through a directional output nozzle 136 at the top of the bio-air sterilization cabinet 102.

When operated during periods of time when the room is occupied, circulation through the bio-air sterilization system destroys airborne contaminants and filters out particulates that are reintroduced into the environment. The air emitted from the present bio-air sterilization system is highly ionized, free of odor, and will continue to control odor, bacteria and viruses while the system is in operation.

High-efficiency particulate air (HEPA),[1] also known as high-efficiency particulate absorbing and high-efficiency particulate arrestance,] is an efficiency standard of air filter.[

Filters meeting the HEPA standard must satisfy certain levels of efficiency. Common standards require that a HEPA air filter must remove—from the air that passes through—at least 99.95%

(European Standard)[4] or 99.97% (ASME, U.S. DOE) of particles whose diameter is equal to 0.3 μm; with the filtration efficiency increasing for particle diameters both less than and greater than 0.3 μm.[7] See the Mechanism and Specifications sections for more information.

HEPA was commercialized in the 1950s, and the original term became a registered trademark and later a generic term for highly efficient filters.[8] HEPA filters are used in applications that require contamination control, such as the manufacturing of disk drives, medical devices, semiconductors, nuclear, food and pharmaceutical products, as well as in hospitals,[9] homes and vehicles.

HEPA filters are composed of a mat of randomly arranged fibres.] The fibers are typically composed of fiberglass and possess diameters between 0.5 and 2.0 micrometers. Key factors affecting its functions are fiber diameter, filter thickness, and face velocity. The air space between HEPA filter fibers is typically much greater than 0.3 μm. The common assumption[that a HEPA filter acts like a sieve where particles smaller than the largest opening can pass through is incorrect and impractical. Unlike membrane filters at this pore size, where particles as wide as the largest opening or distance between fibers can not pass in between them at all, HEPA filters are designed to target much smaller pollutants and particles. These particles are trapped (they stick to a fiber) through a combination of the following three mechanisms:

Diffusion

An enhancing mechanism that is a result of the collision with gas molecules by the smallest particles, especially those below 0.1 μm in diameter, which are thereby impeded and delayed in their path through the filter; this behavior is similar to Brownian motion and raises the probability that a particle will be stopped by either interception or impaction; this mechanism becomes dominant at lower air flow

Interception

Particles following a line of flow in the air stream come within one radius of a fiber and adhere to it.

Impaction

Larger particles are unable to avoid fibers by following the curving contours of the air stream and are forced to embed in one of them directly; this effect increases with diminishing fiber separation and higher air flow velocity.

Diffusion predominates below the 0.1 μm diameter particle size, whilst impaction and interception predominate above 0.4 μm. In between, near the most penetrating particle size (MPPS) 0.21 μm, both diffusion and interception are comparatively inefficient.[Because this is the weakest point in the filter's performance, the HEPA specifications use the retention of particles near this size (0.3 μm) to classify the filter However it is possible for particles smaller than the MPPS to not have filtering efficiency greater than that of the MPPS. This is due to the fact that these particles can act as nucleation sites for mostly condensation and form particles near the MPPS.

Gas filtration

HEPA filters are designed to arrest very fine particles effectively, but they do not filter out gasses and odor molecules. Circumstances requiring filtration of volatile organic compounds, chemical vapors, cigarette, pet, and/or flatulence odors call for the use of an activated carbon (charcoal) or other type of filter instead of or in addition to a HEPA filter. Carbon cloth filters, claimed to be many times more efficient than the granular activated carbon form at adsorption of gaseous pollutants, are known as HEGA filters ("High Efficiency Gas Adsorption") and were originally developed by the British military as a defense against chemical warfare

Pre-filter and HEPA filter

A HEPA filter can be used in conjunction with a pre-filter (usually carbon-activated) to extend the usage life of the more expensive HEPA filter.[In such setup, the first stage in the filtration process is made up of a pre-filter which removes most of the larger dust, hair, PM10 and pollen particles from the air. The second stage high-quality HEPA filter, which filters out the finer particles that escapes from the pre-filter.

Specification

HEPA filters, as defined by the United States Department of Energy (DOE) standard adopted by most American industries, remove at least 99.97% of airborne particles 0.3 micrometers (μm) in diameter. The filter's minimal resistance to airflow, or pressure drop, is usually specified around 300 pascals (0.044 psi) at its nominal volumetric flow rate.

The specification used in the European Union: European Standard EN 1822-1:2009, defines several classes of HEPA filters by their retention at the given most penetrating particle size (MPPS). The averaged efficiency of the filter is called "overall", and the efficiency at a specific point is called "local

Ultraviolet germicidal irradiation

Ultraviolet germicidal irradiation (UVGI) is a disinfection method that uses short- wavelength ultraviolet (ultraviolet C or UVC), light to kill or inactivate microorganisms by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions.UVGI is used in a variety of applications, such as food, air, and water purification.

UVC light is weak at the Earth's surface as the ozone layer of the atmosphere blocks it. UVGI devices can produce strong enough UVC light in circulating air or water systems to make them inhospitable environments to microorganisms such as bacteria, viruses, molds and other pathogens. UVGI can be coupled with a filtration system to sanitize air and water.

The application of UVGI to disinfection has been an accepted practice since the mid-20th century. It has been used primarily in medical sanitation and sterile work facilities. Increasingly, it has been employed to sterilize drinking and wastewater, as the holding facilities are enclosed and can be circulated to ensure a higher exposure to the UV. In recent years UVGI has found renewed application in air purifiers.

UV light is electromagnetic radiation with wavelengths shorter than visible light but longer than X-rays. UV can be separated into various ranges, with short-wavelength UV (UVC) considered "germicidal UV". Wavelengths between about 200 nm and 300 nm are strongly absorbed by nucleic acids. The absorbed energy can result in defects including pyrimidine dimers. These dimers can prevent replication or can prevent the expression of necessary proteins, resulting in the death or inactivation of the organism.

Mercury-based lamps operating at low vapor pressure emit UV light at the 253.7 nm line.

Ultraviolet light-emitting diodes (UVC LED) lamps emit UV light at selectable wavelengths between 255 and 280 nm.

Pulsed-xenon lamps emit UV light across the entire UV spectrum with a peak emission near 230 nm.

This process is similar to the effect of longer wavelengths (UVB) producing sunburn in humans. Microorganisms have less protection against UV, and cannot survive prolonged exposure to it.

A UVGI system is designed to expose environments such as water tanks, sealed rooms and forced air systems to germicidal UV. Exposure comes from germicidal lamps that emit germicidal UV at the correct wavelength, thus irradiating the environment. The forced flow of air or water through this environment ensures exposure.

Effectiveness

The effectiveness of germicidal UV depends on the length of time a microorganism is exposed to UV, the intensity and wavelength of the UV radiation, the presence of particles that can protect the microorganisms from UV, and a microorganism's ability to withstand UV during its exposure.

In many systems, redundancy in exposing microorganisms to UV is achieved by circulating the air or water repeatedly. This ensures multiple passes so that the UV is effective against the highest number of microorganisms and will irradiate resistant microorganisms more than once to break them down.

"Sterilization" is often misquoted as being achievable. While it is theoretically possible in a controlled environment, it is very difficult to prove and the term "disinfection" is generally used by companies offering this service as to avoid legal reprimand. Specialist companies will often advertise a certain log reduction, e.g., 6-log reduction or 99.9999% effective, instead of sterilization. This takes into consideration a phenomenon known as light and dark repair (photoreactivation and base excision repair, respectively), in which a cell can repair DNA that has been damaged by UV light.

The effectiveness of this form of disinfection depends on line-of-sight exposure of the microorganisms to the UV light. Environments where design creates obstacles that block the UV light are not as effective. In such an environment, the effectiveness is then reliant on the placement of the UVGI system so that line of sight is optimum for disinfection.

Dust and films coating the bulb lower UV output. Therefore, bulbs require periodic cleaning and replacement to ensure effectiveness. The lifetime of germicidal UV bulbs varies depending on design. Also, the material that the bulb is made of can absorb some of the germicidal rays.

Lamp cooling under airflow can also lower UV output; thus, care should be taken to shield lamps from direct airflow, or to add additional lamps to compensate for the cooling effect.

Increases in effectiveness and UV intensity can be achieved by using reflection. Aluminum has the highest reflectivity rate versus other metals and is recommended when using UV

One method for gauging UV effectiveness in water disinfection applications is to compute UV dose. The U.S. EPA publishes UV dosage guidelines for water treatment applications.[10] UV dose cannot be measured directly but can be inferred based on the known or estimated inputs to the process:

Flow rate (contact time)

Transmittance (light reaching the target)

Turbidity (cloudiness)

Lamp age or fouling or outages (reduction in UV intensity)

In air and surface disinfection applications the UV effectiveness is estimated by calculating the UV dose which will be delivered to the microbial population. The UV dose is calculated as follows:

UV dose μWs/cm2 = UV intensity μW/cm2 × exposure time (seconds)

The UV intensity is specified for each lamp at a distance of 1 meter. UV intensity is inversely proportional to the square of the distance so it decreases at longer distances. Alternatively, it rapidly increases at distances shorter than 1m. In the above formula the UV intensity must always be adjusted for distance unless the UV dose is calculated at exactly 1m from the lamp. Also, to ensure effectiveness the UV dose must be calculated at the end of lamp life (EOL is specified in number of hours when the lamp is expected to reach 80% of its initial UV output) and at the furthest distance from the lamp on the periphery of the target area. Some shatter-proof lamps are coated with a fluorated ethylene polymer to contain glass shards and mercury in case of breakage; this coating reduces UV output by as much as 20%.

To accurately predict what UV dose will be delivered to the target the UV intensity, adjusted for distance, coating and end of lamp life, will be multiplied by the exposure time. In static applications the exposure time can be as long as needed for an effective UV dose to be reached. In case of rapidly

moving air, in AC air ducts for example, the exposure time is short so the UV intensity must be increased by introducing multiple UV lamps or even banks of lamps. Also, the UV installation must be located in a long straight duct section with the lamps perpendicular to the air flow to maximize the exposure time.

These calculations actually predict the UV fluence and it is assumed that the UV fluence will be equal to the UV dose. The UV dose is the amount of germicidal UV energy absorbed by a microbial population over a period of time. If the microorganisms are planktonic (free floating) the UV fluence will be equal the UV dose. However, if the microorganisms are protected by mechanical particles, such as dust and dirt, or have formed biofilm a much higher UV fluence will be needed for an effective UV dose to be introduced to the microbial population.

Inactivation of microorganisms

The degree of inactivation by ultraviolet radiation is directly related to the UV dose applied to the water. The dosage, a product of UV light intensity and exposure time, is usually measured in microjoules per square centimeter, or equivalently as microwatt seconds per square centimeter (μW·s/cm2). Dosages for a 90% kill of most bacteria and viruses range from 2,000 to 8,000 μW·s/cm2. Larger parasites such as cryptosporidium require a lower dose for inactivation. As a result, the U.S. Environmental Protection Agency has accepted UV disinfection as a method for drinking water plants to obtain cryptosporidium, giardia or virus inactivation credits. For example, for a 90% reduction of cryptosporidium, a minimum dose of 2,500 μW·s/cm2 is required based on the U.S. EPA UV Guidance Manual published in 2006.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV – Air & Water Microbiology – SMB2203

Wate quality refers to the chemical, physical, biological, and radiological characteristics of water. It is a measure of the condition of water relative to the requirements of one or more biotic species, or to any human need or purpose.] It is most frequently used by reference to a set of standards against which compliance, generally achieved through treatment of the water, can be assessed. The most common standards used to assess water quality relate to health of ecosystems, safety of human contact, and drinking water.

Waterborne diseases are conditions caused by pathogenic micro-organisms that are transmitted in water. These diseases can be spread while bathing, washing, drinking water, or by eating food exposed to contaminated water. While diarrhea and vomiting are the most commonly reported symptoms of waterborne illness, other symptoms can include skin, ear, respiratory, or eye problems.

Various forms of waterborne diarrheal disease are the most prominent examples, and affect children in developing countries most dramatically. According to the World Health Organization, waterborne diseases account for an estimated 3.6% of the total DALY (disability- adjusted life year) global burden of disease, and cause about 1.5 million human deaths annually. The World Health Organization estimates that 58% of that burden, or 842,000 deaths per year, is attributable to a lack of safe drinking water supply, sanitation and hygiene (summarized as WASH)

The term waterborne disease is reserved largely for infections that predominantly are transmitted through contact with or consumption of infected water. Trivially, many infections may be transmitted by microbes or parasites that accidentally, possibly as a result of exceptional circumstances, have entered the water, but the fact that there might be an occasional freak infection need not mean that it is useful to categorise the resulting disease as "waterborne". Nor is it common practice to refer to diseases such as malaria as "waterborne" just because mosquitoes have aquatic phases in their life cycles, or because treating the water they inhabit happens to be an effective strategy in control of the mosquitoes that are the vectors.

Microorganisms causing diseases that characteristically are waterborne prominently include protozoa and bacteria, many of which are intestinal parasites, or invade the tissues or circulatory system through walls of the digestive tract. Various other waterborne diseases are caused by viruses. (In spite of philosophical difficulties associated with defining viruses as "organisms", it is practical and convenient to regard them as microorganisms in this connection.)

Yet other important classes of water-borne diseases are caused by metazoan parasites. Typical examples include certain Nematoda, that is to say "roundworms". As an example of water-borne Nematode infections, one important waterborne nematodal disease is Dracunculiasis. It is acquired

by swallowing water in which certain copepoda occur that act as vectors for the Nematoda. Anyone swallowing a copepod that happens to be infected with Nematode larvae in the genus Dracunculus, becomes liable to infection. The larvae cause guinea worm disease

Another class of waterborne metazoan pathogens are certain members of the Schistosomatidae, a family of blood flukes. They usually infect victims that make skin contact with the water. Blood flukes are pathogens that cause Schistosomiasis of various forms, more or less seriously affecting hundreds of millions of people worldwide.

Long before modern studies had established the germ theory of disease, or any advanced understanding of the nature of water as a vehicle for transmitting disease, traditional beliefs had cautioned against the consumption of water, rather favouring processed beverages such as beer, wine and tea. For example, in the camel caravans that crossed Central Asia along the Silk Road, the explorer Owen Lattimore noted, The reason we drank so much tea was because of the bad water. Water alone, unboiled, is never drunk. There is a superstition that it causes blisters on the feet.

Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Botulism	*Clostridium botulinum*	Bacteria can enter an open wound from contaminated water sources. Can enter the gastrointestinal tract through consumption of contaminated drinking water or (more commonly) food	Dry mouth, blurred and/or double vision, difficulty swallowing, muscle weakness, difficulty breathing, slurred speech, vomiting and sometimes diarrhea. Death is usually caused by respiratory failure.
Campylobacteriosis	Most commonly caused by *Campylobacter jejuni*	Drinking water contaminated with feces	Produces dysentery-like symptoms along with a high fever. Usually lasts 2–10 days.
Cholera	Spread by the bacterium *Vibrio cholerae*	Drinking water contaminated with the bacterium	In severe forms it is known to be one of the most rapidly fatal illnesses known. Symptoms include very watery diarrhea, nausea, cramps, nosebleed, rapid pulse, vomiting, and hypovolemic shock (in severe cases), at which point death can occur in 12–18 hours.

E. coli Infection	Certain strains of *Escherichia coli* (commonly *E. coli*)	Water contaminated with the bacteria	Mostly diarrhea. Can cause death in immunocompromised individuals, the very young, and the elderly due to dehydration from prolonged illness.
M. marinum infection	*Mycobacterium marinum*	Naturally occurs in water, most cases from exposure in swimming pools or more frequently aquariums; rare infection since it mostly infects immunocompromised individuals	Symptoms include lesions typically located on the elbows, knees, and feet (from swimming pools) or lesions on the hands (aquariums). Lesions may be painless or painful.
Dysentery	Caused by a number of species in the genera *Shigella* and *Salmonella* with the most common being *Shigella dysenteriae*	Water contaminated with the bacterium	Frequent passage of feces with blood and/or mucus and in some cases vomiting of blood.
Legionellosis (two distinct forms: Legionnaires' disease and Pontiac fever)	Caused by bacteria belonging to genus *Legionella* (90% of cases caused	Legionella is a very common organism that reproduces to high numbers in warm water;[1] but only causes	Pontiac fever produces milder symptoms resembling acute influenza without pneumonia. Legionnaires' disease has severe

	by *Legionella pneumophila*)	severe disease when aerosolized	symptoms such as fever, chills, pneumonia (with cough that sometimes produces sputum), ataxia, anorexia, muscle aches, malaise and occasionally diarrhea and vomiting
Leptospirosis	Caused by bacterium of genus *Leptospira*	Water contaminated by the animal urine carrying the bacteria	Begins with flu-like symptoms then resolves. The second phase then occurs involving meningitis, liver damage (causes jaundice), and kidney failure
Otitis Externa (swimmer 's ear)	Caused by a number of bacterial and fungal species.	Swimming in water contaminated by the responsible pathogens	Ear canal swells, causing pain and tenderness to the touch
Salmonellosis	Caused by many bacteria of genus *Salmonella*	Drinking water contaminated with the bacteria. More common as a food borne illness.	Symptoms include diarrhea, fever, vomiting, and abdominal cramps
Typhoid fever	*Salmonella typhi*	Ingestion of water contaminated with feces of an infected person	Characterized by sustained fever up to 40 °C (104 °F), profuse sweating; diarrhea may occur.

			Symptoms progress to delirium, and the spleen and liver enlarge if untreated. In this case it can last up to four weeks and cause death. Some people with typhoid fever develop a rash called "rose spots", small red spots on the abdomen and chest.
Vibrio Illness	*Vibrio vulnificus*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus*	Can enter wounds from contaminated water. Also acquired by drinking contaminated water or eating undercooked oysters.	Symptoms include abdominal tenderness, agitation, bloody stools, chills, confusion, difficulty paying attention (attention deficit), delirium, fluctuating mood, hallucination, nosebleeds, severe fatigue, slow, sluggish, lethargic feeling, weakness.

Viruses

Disease and Transmission	Viral Agent	Sources of Agent in Water Supply	General Symptoms
SARS (Severe Acute Respiratory Syndrome)	Coronavirus	Manifests itself in improperly treated water	Symptoms include fever, myalgia, lethargy, gastrointestinal symptoms, cough, and sore throat
Hepatitis A	Hepatitis A virus (HAV)	Can manifest itself in water (and food)	Symptoms are only acute (no chronic stage to the virus) and include Fatigue, fever, abdominal pain, nausea, diarrhea, weight loss, itching, jaundice and depression.
Hepatitis E (fecal-oral)	Hepatitis E virus (HEV)	Enters water through the feces of infected individuals	Symptoms of acute hepatitis (liver disease), including fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, jaundice, dark urine, clay-colored stool, and joint pain
Acute gastrointestinal illness [AGI] (fecal-oral; spread by food, water, person-to-person, and fomites)	Norovirus	Enters water through the feces of infected individuals	Diarrhea, vomiting, nausea, stomach pain
Poliomyelitis (P olio)	Poliovirus	Enters water through	90-95% of patients show no symptoms, 4-8% have minor symptoms (comparatively)

		the feces of infected individuals	with delirium, headache, fever, and occasional seizures, and spastic paralysis, 1% have symptoms of non-paralytic aseptic meningitis. The rest have serious symptoms resulting in paralysis or death
Polyomavirus infection	Two of Polyomavirus : JC virus and BK virus	Very widespread, can manifest itself in water, ~80% of the population has antibodies to Polyomavirus	BK virus produces a mild respiratory infection and can infect the kidneys of immunosuppressed transplant patients. JC virus infects the respiratory system, kidneys or can cause progressive multifocal leukoencephalopathy in the brain (which is fatal).

Algae

Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Desmodesmus infection	desmodesmus armatus	Naturally occurs in water. Can enter open wounds.	Similar to fungal infection.

Parasitic worms

Disease and Transmission	Agent	Sources of Agent in Water Supply	General Symptoms
Dracunculiasis [Guinea worm disease] (ingestion of contaminated water)	*Dracunculus medinensis*	Female worm emerges from host skin and releases larvae in water	Slight fever, itchy rash, nausea, vomiting, diarrhea, dizziness, followed by formation of painful blister (typically on lower body parts)

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UNIT –V – Air & Water Microbiology – SMB2203

Bacteriological water analysis is a method of analysing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe to use.

The interpretation and the action trigger levels for different waters vary depending on the use made of the water. Whilst very stringent levels apply to drinking water, more relaxed levels apply to marine bathing waters, where much lower volumes of water are expected to be ingested by users

The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern. Indicator organisms are bacteria such as non-specific coliforms, Escherichia coli and Pseudomonas aeruginosa that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage. Indicator organisms are used because even when a person is infected with a more pathogenic bacteria, they will still be excreting many millions times more indicator organisms than pathogens. It is therefore reasonable to surmise that if indicator organism levels are low, then pathogen levels will be very much lower or absent. Judgements as to suitability of water for use are based on very extensive precedents and relate to the probability of any sample population of bacteria being able to be infective at a reasonable statistical level of confidence.

Analysis is usually performed using culture, biochemical and sometimes optical methods. When indicator organisms levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (where suspected) using specific culture methods or molecular biology

The most reliable methods are direct plate count method and membrane filtration method. mEndo Agar is used in the membrane filtration while VRBA Agar is used in the direct plate count method. VRBA stands for violet red bile agar. A media that contains bile salts which promotes the growth of gram negative and has inhibitory characteristic to gram positive although not complete inhibitory. These media contain lactose which is usually fermented by lactose fermenting bacteria producing colonies that can be identified and characterised. Lactose fermenting produce colored colonies while non lactose fermenting produce colorless ones. Because the analysis is always based on a very small sample taken from a very large volume of water, all methods rely on statistical principles.[2]

Multiple tube method

One of the oldest methods is called the multiple tube method.[3] In this method a measured sub-sample (perhaps 10 ml) is diluted with 100 ml of sterile growth medium and an aliquot of 10 ml is then decanted into each of ten tubes. The remaining 10 ml is then diluted again and the process repeated. At the end of 5 dilutions this produces 50 tubes covering the dilution range of 1:10 through to 1:10000.

The tubes are then incubated at a pre-set temperature for a specified time and at the end of the process the number of tubes with growth in is counted for each dilution. Statistical tables are then used to derive the concentration of organisms in the original sample. This method can be enhanced by using indicator medium which changes colour when acid forming species are present and by including a tiny inverted tube called a Durham tube in each sample tube. The Durham inverted tube catches any gas produced. The production of gas at 37 degrees Celsius is a strong indication of the presence of Escherichia coli.

ATP Testing

An ATP test is the process of rapidly measuring active microorganisms in water through detection adenosine triphosphate (ATP). ATP is a molecule found only in and around living cells, and as such it gives a direct measure of biological concentration and health. ATP is quantified by measuring the light produced through its reaction with the naturally occurring enzyme firefly luciferase using a luminometer. The amount of light produced is directly proportional to the amount of biological energy present in the sample.

Second generation ATP tests are specifically designed for water, wastewater and industrial applications where, for the most part, samples contain a variety of components that can interfere with the ATP assay.

Plate count

The plate count method relies on bacteria growing a colony on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on a plate can be counted. To be effective, the dilution of the original sample must be arranged so that on average between 30 and 300 colonies of the target bacterium are grown. Fewer than 30 colonies makes the interpretation statistically unsound whilst greater than 300 colonies often results in overlapping colonies and imprecision in the count. To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured. This approach is widely utilised for the evaluation of the effectiveness of water treatment by the inactivation of representative microbial contaminants such as E. coli following ASTM D5465

The laboratory procedure involves making serial dilutions of the sample (1:10, 1:100, 1:1000, etc.) in sterile water and cultivating these on nutrient agar in a dish that is sealed and incubated. Typical media include plate count agar for a general count or MacConkey agar to count Gram-negative bacteria such as E. coli. Typically one set of plates is incubated at 22 °C and for 24 hours and a second set at 37 °C for 24 hours. The composition of the nutrient usually includes reagents that resist the growth of non-target organisms and make the target organism easily identified, often by a colour change in the medium. Some recent methods include a fluorescent agent so that counting of the colonies can be automated. At the end of the incubation period the colonies are counted by eye, a procedure that takes a few moments and does not require a microscope as the colonies are typically a few millimetres across.

Pour plate method

When the analysis is looking for bacterial species that grow poorly in air, the initial analysis is done by mixing serial dilutions of the sample in liquid nutrient agar which is then poured into bottles which are then sealed and laid on their sides to produce a sloping agar surface. Colonies that develop in the body of the medium can be counted by eye after incubation.

The total number of colonies is referred to as the total viable count (TVC). The unit of measurement is cfu/ml (or colony forming units per millilitre) and relates to the original sample. Calculation of this is a multiple of the counted number of colonies multiplied by the dilution used.

When samples show elevated levels of indicator bacteria, further analysis is often undertaken to look for specific pathogenic bacteria. Species commonly investigated in the temperate zone include

Salmonella typhi and Salmonella Typhimurium. Depending on the likely source of contamination investigation may also extend to organisms such as Cryptosporidium spp. In tropical areas analysis of Vibrio cholerae is also routinely undertaken.

MacConkey agar is culture medium designed to grow Gram-negative bacteria and stain them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose), lactose and peptone. Alfred Theodore MacConkey developed it while working as a bacteriologist for the Royal Commission on Sewage Disposal in the United Kingdom.

Endo agar contains peptone, lactose, dipotassium phosphate, agar, sodium sulfite, basic fuchsin and was originally developed for the isolation of Salmonella typhi, but is now commonly used in water analysis. As in MacConkey agar, coliform organisms ferment the lactose, and the colonies become red. Non-lactose-fermenting organisms produce clear, colourless colonies against the faint pink background of the medium.[

mFC medium is used in membrane filtration and contains selective and differential agents. These include rosolic acid to inhibit bacterial growth in general, except for faecal coliforms, bile salts inhibit non-enteric bacteria and aniline blue indicates the ability of faecal coliforms to ferment lactose to acid that causes a pH change in the medium.

TYEA medium contains tryptone, yeast extract, common salt and L-arabinose per liter of glass distilled water and is a non selective medium usually cultivated at two temperatures (22 and 36 °C) to determine a general level of contamination (a.k.a. colony count).

MPN tune test

Most Probable Number (MPN) is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, agricultural products and is particularly useful with samples that contain particulate material that interferes with plate count enumeration methods.

MPN is most commonly applied for quality testing of water i.e to ensure whether the water is safe or not in terms of bacteria present in it. A group of bacteria commonly referred as fecal coliforms act as an indicator for fecal contamination of water. The presence of very few fecal coliform bacteria would

indicate that a water probably contains no disease causing organisms, while the presence of large numbers of fecal coliform bacteria would indicate a very high probability that the water could contain disease producing organisms making the water unsafe for consumption.

From each of the fermentation tubes with positive results transfer one loopful of medium to:

- 3 ml lactose-broth or brilliant green lactose fermentation tube,
- to an agar slant and
- 3 ml tryptone water

Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen,further incubate up to maximum of 48 ±3 hours to check gas production.

The agar slants should be incubated at 37°C for 24± 2h and Gram-stained preparations made from the slants should be examined microscopically.The formation of gas in lactose broth and the demonstration of Gram negative, non-spore-forming bacilli in the correspnding agar indicates the presence of a member of the coliform group in the sample examined.The absence of gas formation in lactose broth or the failure to demonstrate Gram-negative, non-spore-forming bacilli in the corresponding agar slant constitutes a negative test (absence of coliforms in the tested sample).

Tryptone water Test

01. Incubate the tryptone water at (44.5 ±0.2°C) for 18-24h

02. Following incubation, add approximately 0.1ml of Kovacs reagent and mix gently.

03. The presence of indole is indicated by a red colour in the Kovacs reagent, forming a film over the aqueous phase of the medium.
    01. Confirmatory tests positive for indole, growth, and gas production show the presence of thermotolerant coli.
    02. Growth and gas production in the absence of indole confirm thermotolerant coliforms.

3. Completed test

Since some of the positive results from the confirmatory test may be false, it is desirable to do completed tests. For this inoculum from each positive tube of the confirmatory test is streaked on a plate of EMB or Endo agar. In this process, a loopful of sample from each positive BGLB tubes is streaked onto selective medium like Eosin Methylene Blue agar or Endo's medium. One plate each is incubated at 37°C and another at 44.5± 0.2°C for 24 hours.

High temperature incubation (44.5 ±0.2) is for detection of thermotolerant E.coli.

Following incubation, all plates are examined for presence of typical colonies.

01. Coliforms produce colonies with greenish metallic sheen which differentiates it from non-coliform colonies (show no sheen). Presence of typical colonies on high temperature (44.5 ±0.2) indicate presence of thermotolerant E.coli.

Advantages of MPN

01. Ease of interpretation, either by observation or gas emission
02. Sample toxins are diluted
03. Effective method of analyzing highly turbid samples such as sediments, sludge, mud, etc. that cannot be analysed by membrane filtration.

Disadvantages of MPN:

01. It takes a long time to get the results
02. Results are not very accurate
03. Requires more hardware (glassware) and media
04. Probability of false positives

Membrane filtration technique

In addition to the multiple tube test, a method utilizing the membrane filter has been recognized by the United States Public Health Service as a reliable method for the detection of coliforms in water. These filter disks are 150 micrometers thick, have pores of 0.45 micrometer diameter, and have 80% area perforation. The precision of manufacture is such that bacteria larger than 0.47 micrometer cannot pass through. 80% area perforation facilitates rapid filtration. To test a sample of water, the water is passed through one of these filters. All bacteria present in the sample will be retained directly

on the filter's surface. The membrane filter is then placed on an absorbent pad saturated with liquid nutrient medium and incubated for 22 to 24 hours. The organisms on the filter disk will form colonies that can be counted under the microscope. If a differential medium such as m Endo MF broth is used, coliforms will exhibit a characteristic golden metallic sheen. The advantages of this method over the multiple tube test are (1) higher degree of reproducibility of results;(2) greater sensitivity since larger volumes of water can be used; and (3) shorter time (one-fourth) for getting results. In this experiment, bacteriological analysis of water is one component of drinking water quality analysis. Water is screened for the presence of fecal contamination by testing for the presence of an indicator microorganism.

Indicator microorganisms are ones that have the following properties:

01. The microorganism is not found in water and will be present in the water only when a contamination event has occurred; and
02. The density of the microorganisms present should be proportional to the degree of contamination. E.coli which is a normal flora present in the intestine of humans and animals meets the above requirements so detection of E.coli and/or related bacteria termed "coliforms" is done using many different techniques as a part of bacteriological analysis of water. One of the techniques is membrane filtration method.

Total coliforms and fecal coliforms

They are the members of the family Enterobacteriaceae. Coliforms may include bacteria of the following genera: Escherichia, Enterobacter, Klebsiella, Citrobacter and Serratia. Fecal coliforms are the subset of total coliforms which are found within the digestive tract and shed through feces. Fecal coliforms can ferment lactose with the production of acid and gas at 44.5°C within 24 hours. They are also said to be thermotolerant coliforms as they can grow at higher temperature. Bacteria belonging to the genera Escherichia and Enterobacter are considered as fecal coliforms.

Detection of total coliforms and fecal coliforms

Total coliforms and fecal coliforms are detected using selective and differential culture medium. For the detection of total coliforms mEndo agar LES is used and for fecal coliforms mFC agar is used. On mEndo agar, coliforms will form red colonies with a metallic sheen and on mFC agar, fecal coliforms will form dark blue colonies. This medium contains lactose and a pH indicator (that

changes color when acid is produced due to fermentation of lactose). Membrane filter is incubated at 35°C for 22-24 hours for the detection of total coliforms.

Coliforms typically will produce metallic (golden) sheen, which is due to the extensive production of aldehydes and acid from the fermentation of lactose.Atypical total coliforms colonies appear as dark red, mucoid or have a dark center but without a metallic sheen. E.coli will form colonies with a metallic sheen.This medium contains bile salts which inhibit other bacteria except enteric. It also contains rosolic acid which inhibits bacteria other than fecal coliforms. Aniline blue is used as a pH indicator which gives blue color in acidic pH. Membrane filter is incubated at 44.5°C for 22-26 hours in mFC agar for the detection of fecal coliforms.Fecal coliforms form blue colonies in this medium (acid is produced due to the fermentation of lactose). E.coli will form flat dark blue colonies.

Materials required

Apparatus

01. Incubator(s) or water-bath(s)
02. Membrane filtration apparatus, complete with vacuum source (electrically operated pump, hand-pump or aspirator) and suction flask.
03. Autoclave for sterilising prepared culture media.
04. Boiling-pan or bath (if filtration apparatus is to be disinfected in boiling water between uses).
05. Laboratory balance
06. Racks for bottles of prepared culture media and dilution water. These must fit into the autoclave.
07. Distilling apparatus with storage capacity for at least 5 litres of distilled water.
08. Refrigerator for storage of prepared culture media.
09. Hot-air steriliser for sterilising pipettes and glass or metal Petri dishes.
10. Thermometer for checking calibration of incubator or water-bath.
11. Pipette cans for sterilising pipettes.
12. Boxes for Petri dishes for use in hot-air steriliser.
13. Reusable bottles for culture media.
14. Measuring cylinders, capacity 100 ml and 250 ml.
15. Reusable pipettes, glass, capacity 1 ml and 10 ml.
16. Bottles to contain 9-ml volumes of buffered dilution water.
17. Flasks for preparation of culture media.
18. Wash-bottle.

19. Blunt-edged forceps.
20. Pipette bulbs.
21. Spatula.
22. Container for used pipettes.
23. Brushes for cleaning glassware (several sizes).
24. Fire extinguisher and first-aid kit.
25. Miscellaneous tools.
26. Waste bin.

Consumables

01. Methanol for disinfecting filtration apparatus using formaldehyde gas (unnecessary in the laboratory, but essential if analyses are done in the field). It is essential to use methanol. Ethanol or methylated spirits cannot be substituted.

02. Membrane filters, 0.45 µm pore size and of diameter appropriate for the filtration apparatus being used and complete with absorbent pads.

03. Disinfectant for cleaning laboratory surfaces and a container for discarded pipettes.

04. Culture media (mEndo Agar LES and mFC Agar).

05. Phosphate-buffered dilution water.

06. Petri dishes, glass or aluminium (reusable) or plastic (disposable).

07. Polyethylene bags for wrapping Petri dishes if dry incubator is used.

08. Magnifying lens (as an aid to counting colonies after filters are incubated).

09. Wax pencils for labelling Petri dishes.

10. Autoclave tape.

11. Detergent for cleaning glassware and equipment.

12. Positive control sample (100 ml of water with a 5 ml of a 1:100 dilution of an overnight culture of E.coli)

13. Negative control sample (sterile water)

14. Water samples to be tested: Volume of water needed for testing.

Water sample collection Precautions

01. Water sample must be collected in a sterile bottle
02. Water sample must be representative of the supply from which it is taken.
03. Contamination of the sample must be avoided during and after sampling.
04. The sample should be tested as promptly as possible after collection.
05. If there is a delay in the examination of the sample, it should be stored at a temperature between 0 and 10°C.

Procedure of membrane filtration technique

01. Sterilise the tips of the blunt-ended forceps in a flame and allow them to cool.

02. Carefully remove a sterile membrane filter from its package, holding it only by its edge.

03. Place the membrane filter in the filter apparatus, and clamp it in place. (If the apparatus has been disinfected by boiling, ensure that it has cooled down before inserting the membrane filter.)

04. Mix the sample by inverting its container several times. Pour or pipette the desired volume of sample into the filter funnel. This volume should normally be chosen in the light of previous experience, but suggested volumes are given in Table 1. If the volume to be filtered is less than 10 ml, it should be made up to at least 10 ml with sterile diluent so that the sample will be distributed evenly across the filter during filtration.

05. Apply a vacuum to the suction flask and draw the sample through the filter; disconnect the vacuum.

06. Dismantle the filtration apparatus and remove the membrane filter using the sterile forceps, taking care to touch only the edge of the filter.

07. Remove the lid of a previously prepared mENDO agar LES plate and place the membrane, grid side uppermost, onto the agar. Lower the membrane, starting at one edge in order to avoid trapping air bubbles between membrane and agar. Mark the petri dish with the sample number or other identification. The sample volume should also be recorded. Use a wax pencil or waterproof pen when writing on Petri dishes.

08. Repeat procedure (1 to 8) with the same volume, but place membranes on mFC plates.

09. Incubate mEndo agar LES plates at 35 ± 0.5°C for 22 – 24 hours and mFC plates at 44.5 ± 0.2°C for 22 to 26 hours, all lid side down. In order to maintain the temperature within such a narrow range, a water bath is typically used for incubation of the mFC agar plates. These plates are placed in watertight plastic bags and then submerged in the water bath.

10. After 22 – 24 hours, remove the mEndo agar LES plates from the 35°C incubator and count the colonies that are dark red, mucoid, have a dark center or (more typically) produce a metallic sheen. These are considered to be total coliform colonies.

11. From the mEndo agar LES plates, choose two total coliform colonies that are isolated on the membrane and confirm that they are Gram-negative rods and non-spore formers.

12. After 22 to 26 hours, remove the mFC agar plates from the 44.5°C incubator and count the colonies that have any blue color. These are considered to be fecal coliform colonies.

13. From the mFC agar plates, choose two fecal coliform colonies that are isolated on the membrane. Confirm that they are Gram-negative rods and non-spore formers.

14. Calculate the total and fecal coliform CFU per 100 ml for each sample as described below:

The original density is estimated from the volume of sample filtered (or the volume of dilution and the dilution factor), and the number of colonies counted on the membrane.

As counts are reported per 100 ml of sample (not per ml), the per ml values must be multiplied by a factor of 100.

$$\text{No. of CFU per 100 ml} = \frac{\text{No.of colonies on the membrane}}{\text{volume filtered}} \times 100$$

If the sample is diluted and the volume of the dilution was filtered, the denominator will be (volume (ml) filtered × dilution)