Assessment of Microbial Air Quality

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Indoor air quality (IAQ)

- Indoor air quality (IAQ) is vital to human health because most human activities take place in the indoor environment including: classrooms, offices and factories.
- Microorganisms such as bacterial and fungal spores are major indoor biological air pollutants, accounting for 5-34% of indoor air pollution and are almost always present in all indoor locations due to their ubiquity in the environment and in human beings.
- Sources of microorganisms in air:
  - pupil's own normal flora & number of pupils occupying the area
  - activity of pupils like sneezing, coughing, talking and yawning
  - Materials such as water, files and other stuffs
  - House-keeping activity such as sweeping or using dry dust mops can aerosolize particles that may contain microorganisms

Factors affecting survival of microorganisms in air

- 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.
- Factors that determine the survival of microorganisms with in a bio-aerosol includes:
  - Suspending medium
  - Temperature
  - Relative humidity
  - Oxygen sensitivity
  - Exposure to UV or electromagnetic radiation
- Many vegetative cells not survive for lengthy periods of time in the air unless the relative humidity and other factors are favorable for survival and the organism is enclosed within some protective cover (eg. Dried organic or inorganic matter).

Microorganisms surviving for longer period in the Environment

- Microorganisms that resist drying and radiations
  - Endospores of bacteria
  - Gram positive bacteria
  - Usually pigmented bacteria
  - Capsule forming bacteria
  - Fungal spores
- Microorganisms surviving for long period can be carried to considerable distances via air and still remain viable.
- They may also settle on surfaces and become airborne again as secondary aerosols during certain activities (eg. sweeping and bed making).

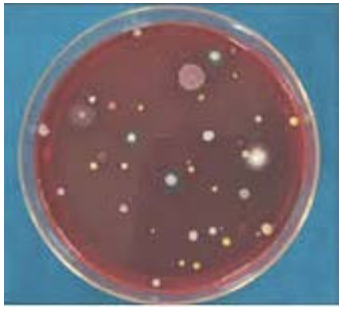
Requirement of microbiological monitoring of air

- 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.

Means of monitoring the microbiological population of the air

- There are two principle means:
  - passive monitoring
  - active sampling
    - Impactors
    - Impingers
- 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



- 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.

- Drawbacks with settle plates:
  - will not detect smaller particles or droplets suspended in the air
  - Not quantitative
  - also vulnerable to interference and contamination from non-airborne sources
  - 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

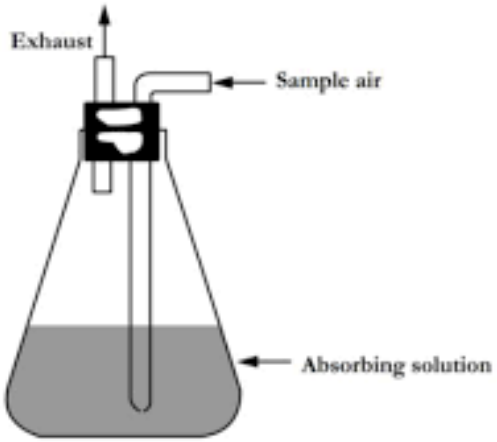
- Plus points of this method:
  - settle plates are inexpensive and easy use, , requiring no special equipment
  - useful for directly monitoring airborne contamination of specific surfaces
  - in an environment such as a low risk food factory, settle plates may provide an adequate means of monitoring 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.

Impingers

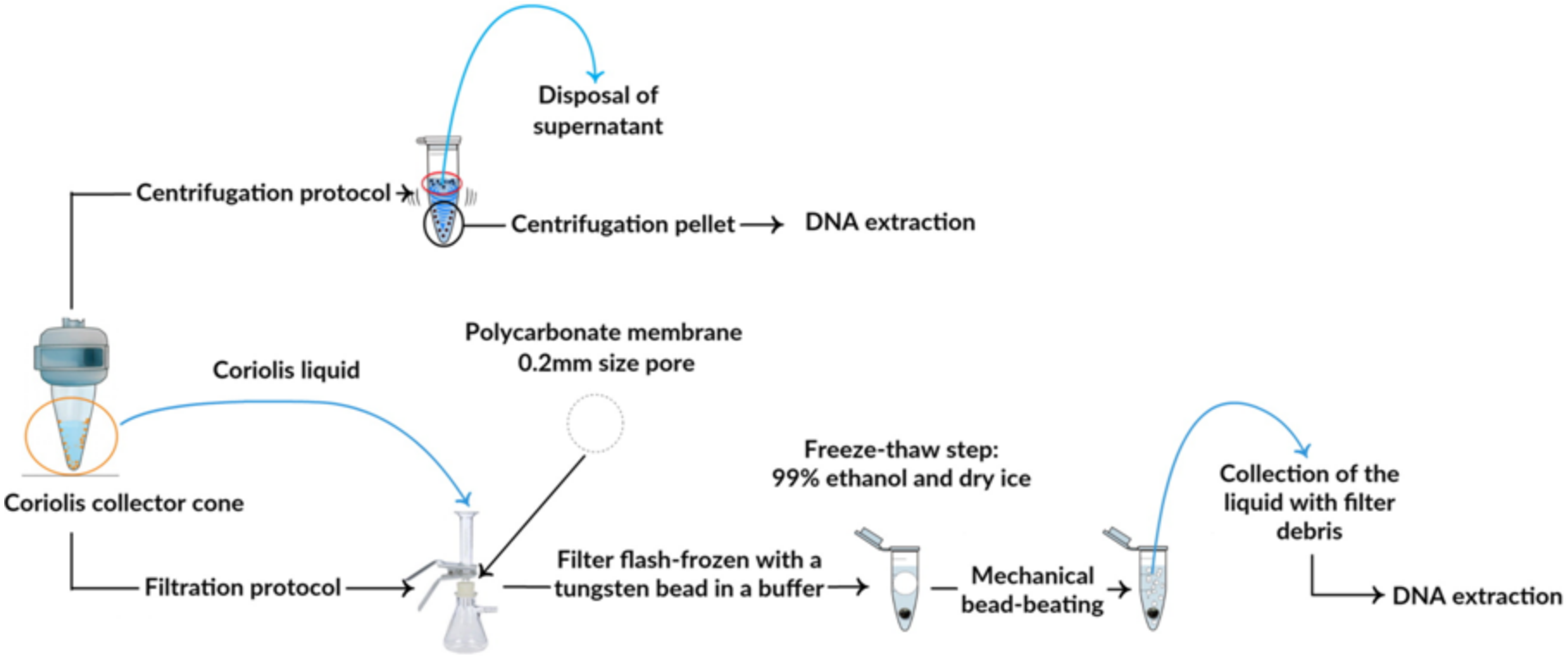
- Impingers use a liquid medium for particle collection.
- Typically, sampled air is drawn by a suction pump through a narrow inlet tube into a small flask containing the collection medium.
- This accelerates the air towards the surface of the collection medium and the flow rate is determined by the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

- Disadvantages:
  - 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
  - overlong sampling times may allow some cells to multiply in the liquid collection medium.
- Advantages:
  - the sample can be analysed using a variety of methods, including molecular techniques such as PCR, so that results can be obtained more rapidly.
- Variations on impinger design: not constructed from glass
  - **Coriolis®µ sampler** made by Bertin Technologies: 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.

Coriolis®µ sampler


Disposal of supernatant
Centrifugation protocol
Centrifugation pellet
DNA extraction
Polycarbonate membrane 0.2mm size pore
Coriolis liquid
Coriolis collector cone
Freeze-thaw step: 99% ethanol and dry ice
Collection of the liquid with filter debris
Filtration protocol
Filter flash-frozen with a tungsten bead in a buffer
Mechanical bead-beating
DNA extraction

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.

...Impactors

- Advantages:
  - 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.
  - Use of a water-soluble polymer gel instead of agar allows the sample to be analysed by rapid techniques such as PCR or cytometry.
- Disadvantage:
  - 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.

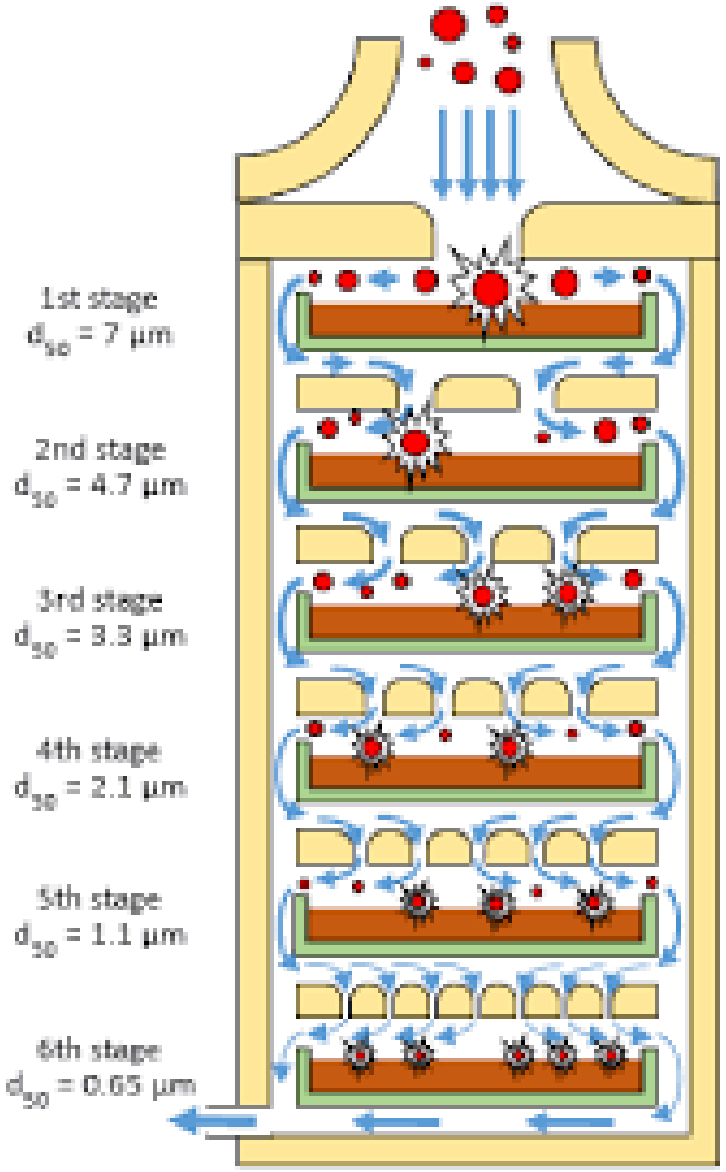
Instruments on Impaction Principle

- 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.

Anderson single stage viable impactor

Anderson two-stage viable impactor

Anderson six-stage viable impactor



Instruments on Impaction Principle

- 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

More recently a number of highly portable and convenient impaction samplers

- Most of these are sieve samplers, such as the **Surface Air System (SAS) samplers** made by pbi International in Italy, and use agar contact plates as the collection surface.
- However, some types, such as the **RCS samplers** from Biotest Diagnostics, 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.
- **Semi-automated systems**, usually based on sieve type impaction samplers.
  - 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.
  - Also possible to set up a wireless network of portable samplers controlled by a central PC, with no need for electrical/ vacuum line connections.

SAS samplers

RCS sampler

Filtration method

- 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.

Air Sampler Validation and Calibration

- Microbiological air sampling usually requires the sampling of large volumes of air (at least 1 m3).
- 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:
  - Physical efficiency of the sampler – the relative efficiency of the sampler in collecting particles over a range of sizes.
  - 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.
  - Validation of the instrument for its intended application and environment.
  - 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.

Questions

- What factors effect the survival of microorganisms in air?
- Write an essay on methods of monitoring of microbial air quality.
- Write down the advantges and disadvantages of various passive and active methods applicable for monitoring of microbial air quality of an environment.
- Write a short note on:
  - Impactors
  - Impingers
  - Passive monitoring