



INDIRECT METHODS OF MEASUREMENT OF MICROBIAL GROWTH


Submitted by,


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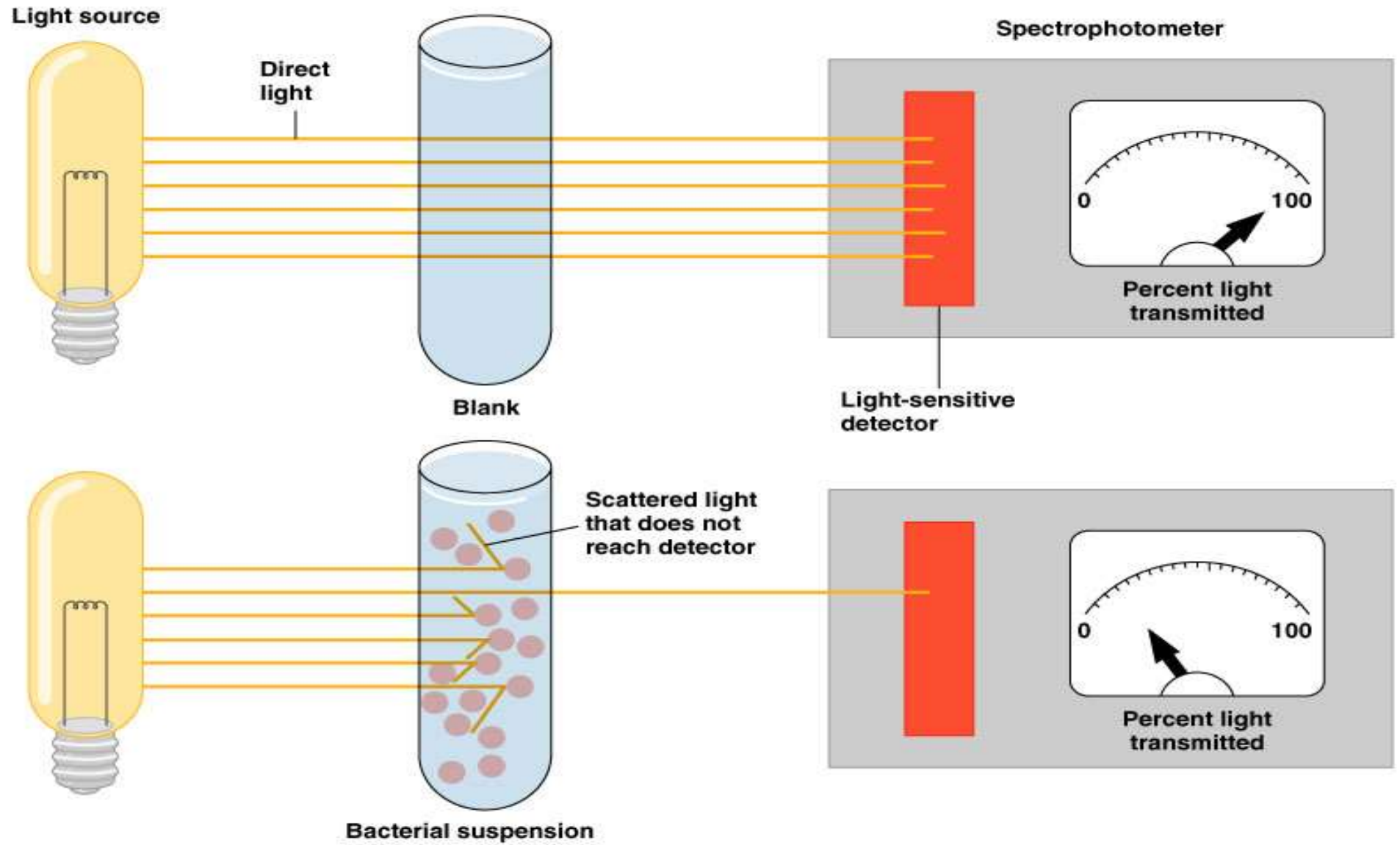


TURBIDITY

- Turbidimetry is a simple, rapid method for following growth.
 - A spectrophotometer or calorimeter can be used for turbidimetric measurements of cell mass.
 - A spectrophotometer is used to determine turbidity ("cloudiness") by measuring the amount of light that passed through a suspension of cells.
 - More cells = more turbidity; more turbidity = less light passing through the suspension
 - However, the culture to be measured must be dense enough to register some turbidity on the instrument.
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- Moreover, it may not be possible to measure cultures grown in deeply coloured media or cultures that contain suspended material other than bacteria.
 - It must also be recognised that dead as well as living cells contribute to turbidity.
 - (% T) percent transmission - fewer cells present (less turbidity) and thus will allow more light to pass through. Therefore the % T is higher when the cell number is lower. Absorbance is the opposite of % T.
 - More light is absorbed when more cells are present and thus absorbance goes up as turbidity (or cell number) goes up.

Turbidimetric method




METABOLIC ACTIVITY

- Another indirect way of estimating bacterial numbers is measuring the metabolic activity of the population (for example, acid production or oxygen consumption, nutrient utilization, waste production, pH, etc).
- The assumption is that the amount of acid produced or oxygen consumed under specific conditions and during a fixed period of time is proportional to the magnitude of bacterial population.
- Admittedly, the measurement of acid or any other end product is a very indirect approach to the measurement of growth and is applicable only in special circumstances.

DRY WEIGHT

- Dry weight of pre-weighed filter paper containing pellets of microbial cells is measured.
- Dry weight of filter paper is nullified by subtracting the dry weight of only filter paper of similar size.
- Thus dry weight of microbial cells can be obtained.
- However, it can be used only with very dense suspensions and the cells must be washed free of all extraneous matter.
- Moreover, dry weight may not always be an indicative of the amount of living material in the cells.

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- Dry weight may continue to increase without corresponding cell growth due to the accumulation of intracellular reserve materials. Yet, for many organisms, the determination of dry weight is an accurate and reliable way to measure growth and is widely used in research.
 - **Advantages**- only way to determine growth of filamentous bacteria. It is rapid and easy.
 - **Disadvantages**- cumbersome, not very accurate. It does not give you cell numbers or increase in mass. It cannot distinguish between live and dead cells and must work within certain absorbency (more than 10^7 and less than 10^8).

IN BRIEF...

These are some of the indirect methods of measuring microbial growth. It help us understand that it is not always necessary to count microbial cells to estimate their numbers.

- In science and industry, microbial numbers and activity are determined by some of the indirect methods as well.
- A spectrophotometer is used to determine turbidity by measuring the amount of light that passes through a suspension of cells.
- An indirect way of estimating bacterial numbers is measuring the metabolic activity of the population (for example, acid production or oxygen consumption).
- For filamentous organisms such as fungi and molds, measuring dry weight is a convenient method of growth measurement.

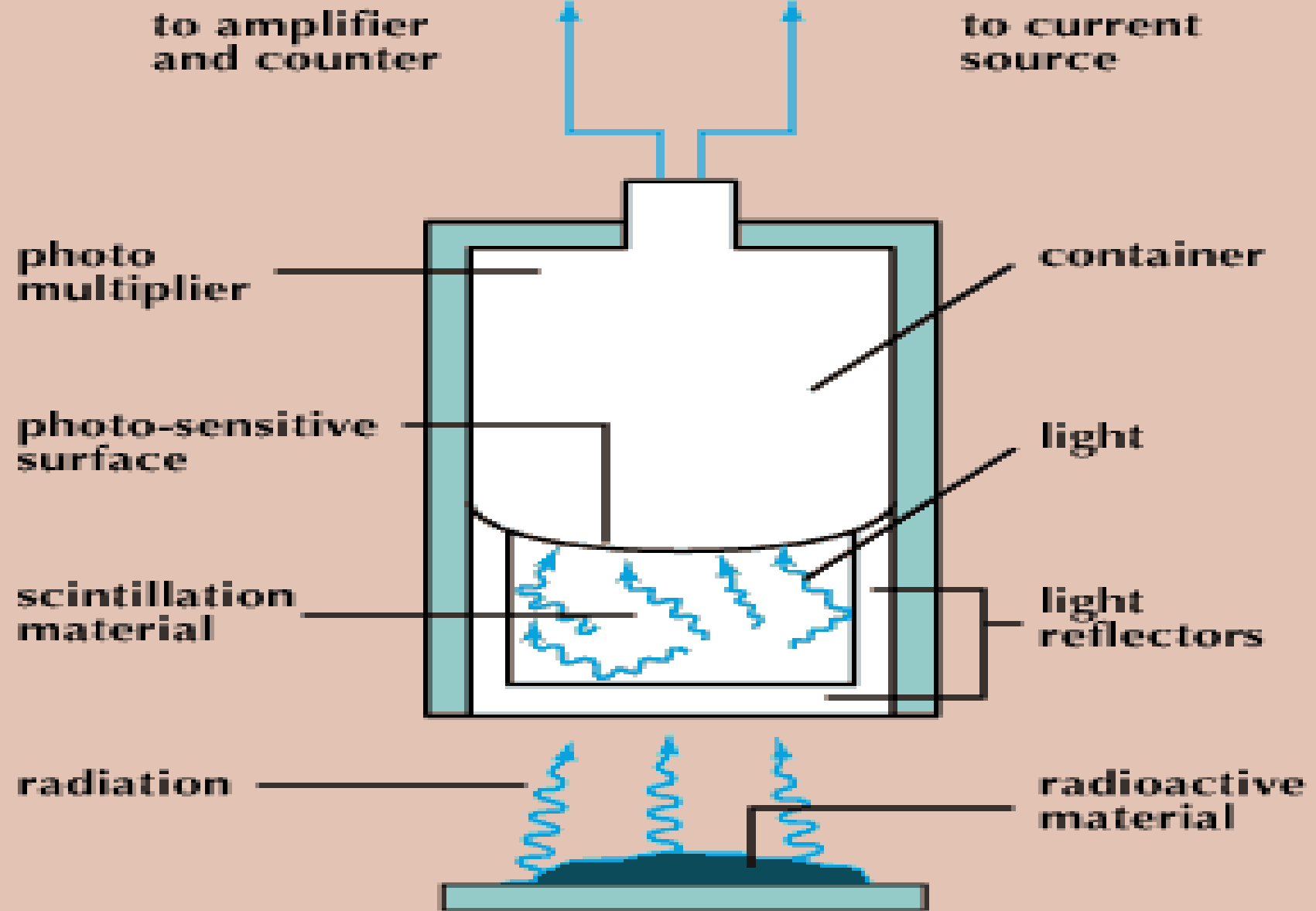


Scintillation counter

Scintillation counter:

- The radio isotopes apart from causing ionisation radiation emit photons of light cause excitation.
- This excitation can be detected and quantified.
- The detection and measurement of this excitation is known as **scintillation** and the instrument which detects the light using a **photo multiplier** is known as **scintillation counter**.
- The electron pulse which is detected by the photomultiplier is a result of conversion of light into electrical energy.
- The modern electronic scintillation counter was invented in 1944 by **Sir. Samuel Curran**.

SCINTILLATION COUNTER



Types of scintillation:

There are **2 types** of scintillation:-

Scintillation can be counted by the two different techniques as follows:-

a) Solid Scintillation (External) counting:-


- In the case, the sample is placed close to a fluor crystal (crystallized silver activated zinc sulphides) for alpha-emitter; sodium iodide for gamma-emitter; anthracene or stilbene for beta-emitters, which in turn is placed adjacent to a photomultiplier. This photo multiplier is connected to a high voltage supply and a scalar.

Solid Scintillation

- Solid scintillation counting is particularly useful for measurement of gamma-emitting isotopes.
- This is so, since the gamma-rays are electromagnetic radiation and only rarely collide with neighbouring atoms to cause ionization or excitation; obviously the densely packed atoms in a crystal provide a better chance of collision.
- On the other hand, solid scintillation counting is not so satisfactory for weak beta-emitters (^3H , ^{14}C , ^{35}S) since even the highest energy negatron have a very low penetrating power.

b) Liquid scintillation counting(Integral):-


- In this case, the radioactive sample is suspended in a scintillation system composed of the solvent and an appropriate scintillator.
- Liquid scintillation counting is extremely useful for quantitating soft beta-emitters.
- The radioactive sample is dissolved or suspended in a scintillation system composed of solvent and primary and secondary scintillators.
- The radiation from the suspended sample molecules collides with a solvent molecule imparting a discrete amount of its energy to the solvent molecule.





Several solvents used in LS counting :- (Ethanol, Acetone, Ethyl glycol, dimethyl ether, 1,4-dioxane, xylene, Methoxybenzene(anisole), Toluene).

Energy transfer in liquid scintillation and subsequent fluorescence excitation occurs as follows:-

- ❖ The solvent molecule, which has become excited as a result of collision with the radiation, emits light as it comes back to ground state, this process is known as **phosphorescence** due to the peculiar chemical nature of the solvent.


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- ❖ The solvent emits light of a very short wavelength which falls in the range of 260-340nm.
 - ❖ This range is too short to be detected by most existing instruments. To circumvent this problem, a **second molecule** is added to the system.
 - ❖ This compound known as **primary fluor**, it absorbs light in the range of 260-340nm. The longer wavelength light can be further increased (for efficient detection) by using a **secondary fluor**.
 - ❖ The secondary fluor absorbs light emitted by the primary fluor and emits light with a maximum in the visible region.

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- Just like the solvent, the fluorescent nature of the primary and the secondary fluors is dependent on their **aromatic nature** and the **availability of pi electrons**.
 - Eg:- Primary fluors :- PPO (2,5-diphenyloxazole)
 - Secondary fluors :- POPOP(1,4-bis-(5-phenyl oxazol-2-yl)-benzene)
 - The amount of light yielded by the fluors is then taken up by photomultiplier which converts light energy into an identical electrical signal that can be easily manipulated and measured.

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- Samples containing same concentration of radioactive sample may produce different no of counts and this discrepancy is expressed in terms of counting efficiency.
 - **Counting efficiency** = $\frac{\text{Counts per minute of the radioactive std}}{\text{Disintegrations/minute of the radioactive std}} * 100$.




Advantages of scintillation counters:

- a) Soft Beta emitters not detectable by GM Counters can be detected with about 50% efficiency.
 - b) Unlike different GM tubes for different sample types, virtually any kind of sample can be accommodated in scintillation counter and its radioactivity can be determined accurately.
 - c) High counter rate are possible
 - d) Sample preparation is relatively easier.
 - e) Chemiluminescence
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Disadvantages:-

- a) Quenching
 - b) Cost per sample of LS counting is significantly higher than GM (Geiger-Muller) counter.
 - c) High voltage applied to the photomultiplier gives rise to electronic events in the system which contribute to high background even.
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Non-radioactive labelling:

- Many years, the use of radiolabels was the method of choice for nucleic acid labelling and detection.
- However, significant improvements to the sensitivity that can be achieved with non-radioactive labelling and detection systems have led to the evolution of a comprehensive range of products that offer rapid, non-destructive alternatives to the use of traditional radioactive techniques.
- Non-radioactive labelling methods are separated into 2 different approaches- direct and indirect.

Non-radioactive labelling methods

➤ Indirect labelling:

- Hapten-based labelling kits such as gene images labelling and detection system introduce nucleotides tagged with fluorescent probe.
- These are then detected with a highly specific anti-fluorescein antibody conjugated to alkaline phosphatase enzyme.

➤ Direct labelling:

- These are alternatives to indirect method.
- It offers significant improvements in speed and convenience.

Conclusion


- Scintillation counters are used to measure radiation in a variety of applications including personnel and environmental monitoring for radioactive contamination, medical imaging, radiometric assay and nuclear plant safety.
- Several products have been introduced in the market utilising scintillation counters for detection of potentially dangerous gamma-emitting materials during transport.
- These include scintillation counters designed for border security, ports, weigh bridge applications, scrap metal yards and contamination monitoring of nuclear waste.
- There are variants of scintillation counters mounted on pick-up trucks and helicopters for rapid response in case of a security situation due to dirty bombs or radioactive waste.

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Previous year questions from this topic:

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1. Turbidimetry
 2. Scintillation counter
 3. Different methods to measure microbial growth

THANK YOU



Submitted to,
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