**Course 2 : Methods of Measuring Microbial Growth**

**Lien avec l’enseignement de LV:**

Synthèse de texte sous forme de mind-map

**La séance d’avant :**

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| **Activité** | **Temps** | **Activité prof** | **Activité élève** | **Document/support** |
| 1. ppt | 30 minutes | Explique le contexte : élèves passent l’épreuve d’ETLV 1. D’où nécessité de travail en autonomie pendant ce temps en salle. |  | Course 2 |
| 2. Mindmap | 15 minutes | Passe les consignes de travail.Pose le début du mind-map en utilisant l’exemple du Total cell count. | Prends des notes sur les consignes et sur le début du mind-map. |  |

Temps prévu : 2h en autonomie.

**Course 2 : Methods of Measuring Microbial Growth**

You will work on the joined document (pp. 2 to 5) making a presentation of methods used to measure bacterial growth.

Carefully read the text.

1. Make a table using Microsoft Word with two columns: column one with the highlighted words, column two with a translation of these words into French.
2. There is a method called the Copacabana method. Find out what it is and give the French name for this method. Match this method with the appropriate category of methods of the joined text. Explain the name Copacabana.

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1. Find on internet a picture illustrating each method. Paste them in the text.
2. Write a short description of the method of the most probable number (paragraph g).
3. Construct a mind map about the measurement of bacterial growth using the text. For each method you can present the following elements using key-words: how the method works, applications, inconvenients (drawbacks), advantages, what you need…

There are different methods of counting microbial growth. These are based on different parameters of cells.

**(a)** **Wet Weight Measurement**

Measuring cell mass is an easy step of cell growth measurement. A known volume of culture sample is centrifuged. The wet weight of pellets is measured by using pre-weighed filter paper. A pre-weighed filter paper of similar size is used to subtract the weight of wet filter paper. Thus wet weight of cells is calculated.

**(b)** **Dry Weight Measurement**

Dry weight measurement of cell material is similar to that of wet weight. Here 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.

**(c)** **Turbidity measurement**

Absorbance is measured by using a spectrophotometer. Scattering of light increases with increase in cell number. Therefore, transmission of light declines. At a particular wavelength absorbance of light is proportional to the cell concentration of bacteria present in the suspension.

A standard graph (between absorbance and cell concentration) can be prepared.

**(d)** **Total Cell Count**

Cell growth is also measured by counting total cell number of the microbes present in that sample. Total cells (both live and dead) of liquid sample are counted by using a special microscope glass slide called Petroff-Hausser Counting Chamber.

In this chamber a grid is marked on the surface of the glass slide with squares of known area. The whole grid has 25 large squares, a total area of 1 mm**2** and a total volume of 0.02 mm**3** (1/50 mm).

All cells are counted in large square and total number per ml sample is measured. If 1 square contains 12 cells, the total number of cells per ml sample will be: 12 cells x 25 square x 50x10**3** = 1.5xl0**7** cells.

**(e) Viable Cell Count**

A viable cell is defined as a cell which is able to divide and increase cell numbers. The normal way to perform a viable cell count is to determine the number of cells in the sample which is capable of forming colonies on a suitable medium. It is assumed that each viable cell will form one colony. Therefore, viable count is often called plate count or colony count. There are three ways of forming plate count.

* **Spread Plate Method**

A volume of culture (0.1 ml) is spread over the surface of an agar plate by using a sterile glass spreader or glass beads. The plate is incubated to develop colonies. Then the number of colonies is counted.

* **Pour Plate Method**

In this method a known volume (0.1-1.0 ml) of the culture is poured into the sterile Petri dishes. Then melted agar medium is poured and mixed gently. The plate is incubated. Colonies growing on the surface of agar are counted.

* **Membrane filtration**

The culture medium Is filtered through a membrane. This membrane is placed onto an agar-plate. The colonies are counted.

**(f) Cell Counter**

It is an electronic device. The microbial culture is directly used to count cells present in the suspension.

**(g) Method of the most probable number**

**More…**

|  |  |
| --- | --- |
| Total Cell Count | Rapid, but at least 10^7 cells must be present to be effectively counted. Counts include living and dead cells. Enumeration of bacteria in milk or cellular vaccines |
| Cell-counting instruments | Coulter counters and flow cytometers count total cells in dilute solutions. Flow cytometers can also be used to count organisms to which fluorescent dyes or tags have been attached. |
| Viable Cell Counts | Used to determine the number of viable bacteria in a sample, but only includes those that can grow in given conditions. Requires an incubation period of aprrox. 24 hours or longer. Selective and differential media can be used to enumerate specific species of bacteria. Enumeration of bacteria in milk, foods, soil, water, laboratory cultures, etc. |
| Plate Count | Time-consuming but technically simple method that does not require sophisticated equipment. Generally used only if the sample has at least 10^2 cells/mL. |
| Membrane Filtration | Concentrates bacteria by filtration before they are plated; thus can be used to count cells in dilute environments. |
| Most Probable Number | Statistical estimation of likely cell number; it is not a precise measurement. Can be used to estimate numbers of bacteria in relatively dilute solutions. |
| Turbidity | Very rapid method; used routinely. A correlation with plate counts is required in order to determine cell number. Estimations of large numbers of bacteria in clear liquid media and broths |
| Total Weight | Tedious and time-consuming. It is one of the best methods for measuring the growth of filamentous microorganisms. Measurement of total cell yield in cultures  |