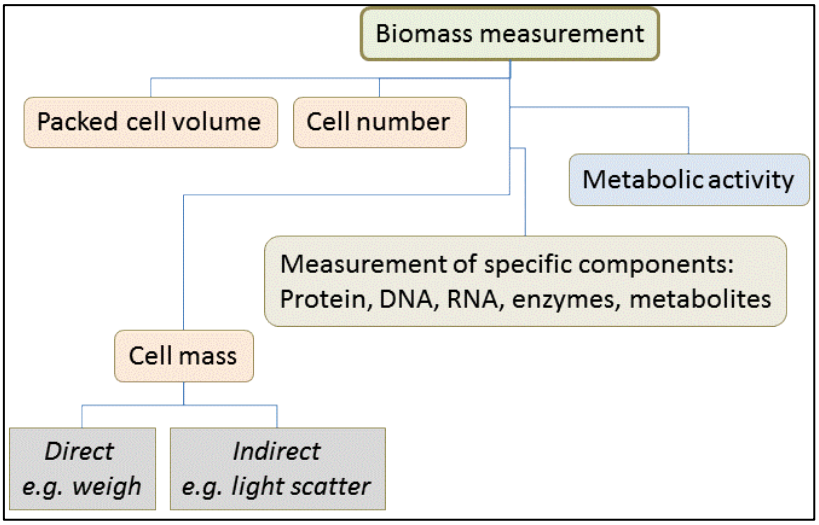
Methods for Estimating Biomass.

There are many methods for estimating biomass, which reflect the requirements of the users of the data. Methods based on the determination of specific components or on metabolic activity often make the assumption that there is a direct correlation between them and the total amount biomass. This is not always valid.



Therefore you should report biomass concentrations based on the determination of cell constituents in such a manner that the basis of the estimation is known, e.g. g.cellular protein cm^{-3}. A few of the more common methods are listed below.

1.1 Measurement of cell mass

1.1.1 Dry Weight Determination

Three stages are involved:

- Separating the organism from the medium, by filtration or by centrifugation
- Washing the cells with a near isotonic saline to avoid cell lysis, but allowing the dry weight of the salt to present after drying.
- Drying the biomass at 80° C for 24 hours or at 110 ° C for 8 hours. It is also possible to use a microwave oven to determine dry weight within 30 min.. Drying and weighing cycles should be repeated until a constant weight is obtained. NB. It is important to cool the sample in a desiccator prior to weighing because the dried biomass is hygroscopic and will begin to take up water from the atmosphere.

The main limitation of the dry weight measurement is that it is relatively insensitive and inaccurate – it is difficult to way with accuracy less than 1 mg, but this dry weight. This may represent as many as 5 billion bacteria. For accuracy, you need to measure around 50 mg of cells or more.

1.1.2 Wet weight determination.

As above except that cells are not dried. Wet weight will include both intracellular and extracellular water. Extracellular water volume can be significant. In *E. coli* the extracellular water volume of close-packed cells has been estimated to be about 10% of total volume, and water represents 75% of the total cell weight. Therefore, for wet weight measurements to be of any use, the centrifugation or filtration method used to pack down the biomass must be carefully standardised.

1.1.3 Turbidity.

Cells scattered light and because cells in a population are of roughly constant size, the amount of scattering is proportional to the concentration of cells present. As a cell suspension increases in concentration it becomes more turbid and less light is transmitted through the medium. The extent of light scattering can be measured using a spectrophotometer and is almost linearly related to concentration at low concentrations and low absorbance levels.

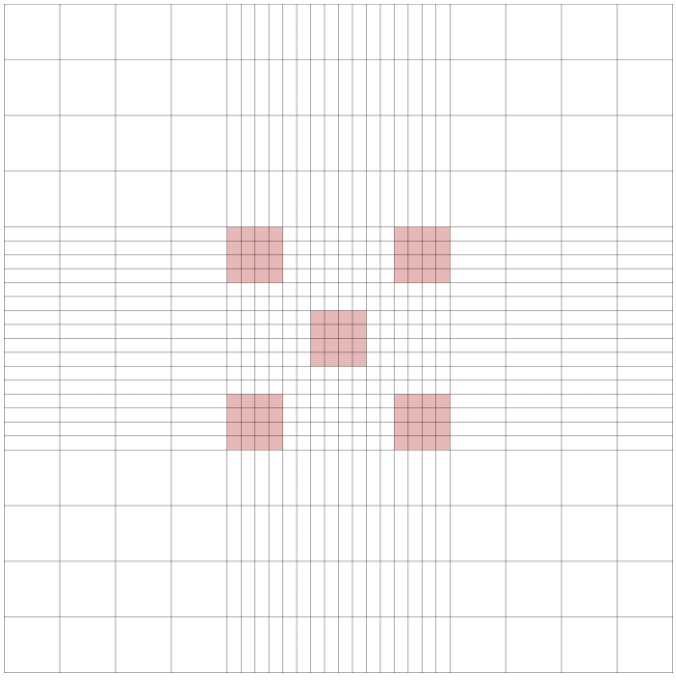
Absorbance (A) is defined as the logarithm of the ratio of intensity of light striking the suspension (I_o) to that transmitted by the suspension (I):

$$A = \log I_0/I$$

To use the turbidity of a culture to measure cell mass, plot a standard curve to show the relationship between turbidity (absorbance at 540 nm) of several dilutions of culture and dry weight of the cell material present.

- Note that a calibration curve prepared for one organism may not be appropriate for another: the amount of light scattered by a cell suspension depends not only on the amount of cell mass present, but also on the size and shape of the cells.
- As the number of cells in the suspension increases a greater proportion of cells do not contribute to light scattering. (The relationship between absorbance (turbidity) and cell mass follows the Beer - Lamberts type relationship)
- it is also possible to plot a graph of absorbance against cell number

1.2 Measurement of Cell Number



Haemocytometer chambers can be used to count the number of cells in the sample under a microscope. These specially designed slides have chambers of known depth with an etched grid on the chamber bottom.

Each small square in the grid shown in the figure has the dimensions 0.05 mm x 0.05 mm and when the coverslip is applied the counting chamber has a depth of 0.1 mm. Each large square has 16 small squares.

Count the number of cells in 5 squares. (Decide beforehand if you count cells on the line or not)

Volume of 5 large squares = 0.05 x 0.05 x 0.1 x 16 x 5 mm^3

Correct for the number of cells per cm^3

By way of example, if 5 squares had a total of 40 cells, the number of cells per ml (cm^3) would be:

$$\frac{40 \times 400 \times 10 \times 1000}{16 \times 5} = 2.0 \times 10^6 \text{ cells } cm^{-3}$$

- For a reasonable count a minimum of 5 cells per large square is required.

- Accuracy depends upon producing and evenly distributed suspension of cells, so the chamber must be clear and free from contaminants such as oil.
- The distribution of cells is random, so the more cells that are counted the more accurate the determination is likely to be. This can be determined statistically but a good simple approximation is that the likely accuracy is reflected by the relationship $N \pm \sqrt{N}$ where N is the number of cells counted. This is quite satisfactory in getting an approximate idea of the likely accuracy of the cell count. Thus, if 49 cells are counted the likely accuracy is ± 7 (i.e. the error could be of the order of 14%).
- A common practice is to stain translucent cells prior to placing them in the counter chamber. This makes them easier to see and therefore easier to count. It also allows cellular material and non-cellular solids to be distinguished.
- If the cells clump, they need to be separated by agitation or the addition of surfactants. In the case of plant cells, dilute chromic acid is often used. But care must be taken to separate cells not to disrupt them.

1.3 Viable cell counts- pore and spread plates.

A different technique is required to determine the number of living viable cells present in a suspension.

In the spread plate technique, a small sample (usually 0.1) is aseptically spread over the surface of an agar plate containing an appropriate medium.

In the pore plate technique, the sample is mixed with melted agar and the mixture poured into a sterile plate. The organisms are thus fixed within the agar gel and form colonies. Larger sample volumes can be used as well as heavy slurries or suspensions. A potential source of error is that organisms may be inactivated by the brief heating in the melted agar (around 48°C) and will not grow and therefore are not counted.

- Each viable cell in a suspension is assumed to give rise to a single colony after incubation on a suitable medium under favourable conditions. After incubation the number of colonies formed is counted and used to estimate the number of viable cells in the original suspension. This technique is predominantly applicable to unicellular microorganisms.
- Only those cells that can multiply under conditions will be counted.
- Not all cells give rise to a colony, because certain cells have a tendency to clump or aggregate: the clumps will give rise to only one colony, regardless of how many cells are in the clump.
- Results are expressed as colony-forming units (CFU) cm^{-3}, rather than cells cm^{-3}.
- Usually only plates containing 30 to 300 colonies are counted to enhance the accuracy of the count. If too crowded, colonies will grow into each other to form more or less confluent growth. If too dilute the results will not be statistically valid.

A problem associated with viable counting is the need for a long incubation time - up to 1 to 2 days.