Methods for measuring the growth of microorganisms

We have seen that the growth of a culture of unicellular microorganisms • results from an increase in the number of cells, so growth of microbial - populations can be measured by determining changes in either the number of cells, or the cell mass. The number of cells in a suspension can be determined by counting the number of cells present in an accurately determined, very small volume of culture medium. This is usually carried out using special microscope slides, known as counting chambers (Counting cells using a haemocytometer). These are slides which are ruled with a grid of squares of known area and are made so that when correctly filled, they contain a film of liquid of known depth. The volume of liquid overlying each square is therefore known. This method for determining cell numbers is referred to as a total cell count, which includes both viable and non-viable cells, as it is not normally possible to distinguish one from another using a microscope.

The number of cells can also be determined using a plate count *(Counting cells using the pour plate dilution method)*. This method depends on the ability of each single, viable cell to grow in or on an agar medium and produce a visible colony. This method of counting is referred to as a viable count, as only those cells which are able to grow in the culture medium are detected. Appropriate dilutions of a bacterial culture are made and are used to. inoculate a suitable medium. The number of viable cells present in the original' culture is then determined by counting the number of colonies which develop after incubation of the plates, and multiplying this number by The dilution factor. Two or three replicate plates of each dilution should be prepared, to reduce the sampling error. The greatest accuracy is obtained with relatively large numbers of colonies on each plate, but the practical limit is reached with between 300 and 400 colonies per plate.

The only direct method for determining cell mass is to measure the dry mass of cells in a known volume of culture medium. This is a suitable method for measuring the growth of a filamentous organism, where cell counting is inappropriate, but it is rarely used for unicellular bacteria, because of the relatively insensitive method for weighing. It is difficult to weigh with an accuracy of less than 1 mg, but this represents the dry mass of between 1 and 5×10^9 bacteria. .

One useful approach for estimating the number of cells present in a suspension is to use an optical method, by determining the amount of light which is scattered by a cell suspension. A suspension of cells appears cloudy, or turbid, to the eye because the cells scatter light passing through the suspension. The cloudiness increases as the cell numbers increase and, within limits, the amount of light scattered by the cells is proportional to their numbers. A colorimeter is an instrument which can be used to measure the amount of light which is transmitted by a cell suspension. When a beam of light passes through a cell suspension, the reduction in the amount of light transmitted gives a measure of cell density. A colorimeter can be calibrated, by combining measurements of light transmitted with another method for- measuring cell growth, such as plate counting.

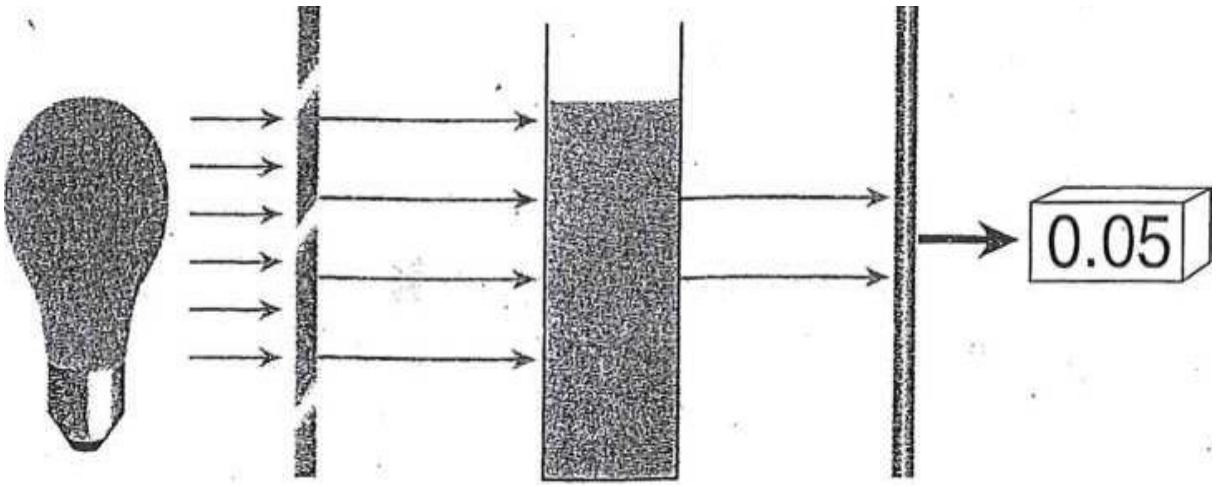

Figure:1 Principle of a colorimeter used to measure cell growth

Methods for culturing microorganisms

If we wish to study a single species of microorganism, it is often necessary to isolate it from a mixed culture of many different species. Microorganisms are present in almost every habitat, soil and water are particularly rich sources, and in order to obtain a pure culture of a single species, it must be grown in a laboratory in suitable conditions, with all the necessary nutrients provided. It is also essential to avoid contaminating the culture with other, unwanted microorganisms. The medium used to grow the microorganism must be sterile, and it is essential to take precautions in handling the materials used for culture of the organism in order to avoid contamination.

Aseptic technique is the term used describe the proper handling of cultures, sterile apparatus and sterile media to prevent contamination. The method used to pour sterile agar medium into Petri dishes.

Transfer of cultures from a mixed broth culture onto an agar plate, is usually carried out using a bacteriological loop which has been sterilized by heating until it is red hot using a

Bunsen burner. If we wish to isolate a fungus from a mouldy tomato, a mounted needle can be used, sterilized in the same way as the loop. The needle is then used to transfer some of the fungal material, including spores, from the tomato to the centre of a Petri dish containing a suitable medium, such as malt extract agar.

Microbiological media are formulated to contain all the nutrients required by particular microorganisms. Some species of microorganism are particularly fastidious in their requirements and their media, are therefore complex, containing a wide range of mineral salts, amino acids, purines, pyrimidines, vitamins and other organic growth factors. Other microorganisms will grow in relatively simple media containing an energy source, usually glucose, and a number of mineral salts. Different microorganisms therefore have different nutrient requirements and for the successful culture of a particular species it is necessary to provide all the essential nutrients, in the correct proportions, in the culture media.

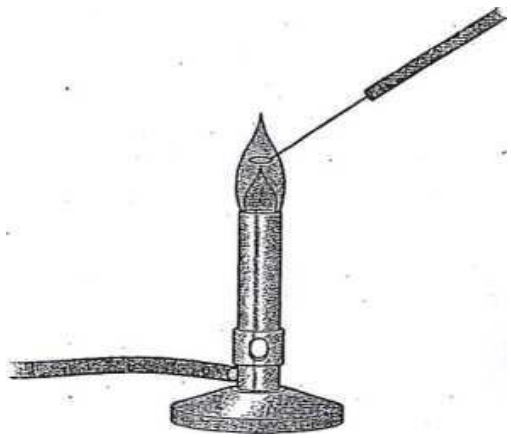

Figure : 2 Sterilizing a bacteriological loop. The loop should be held in the flame until it is red hot, then allowed to cool briefly before being used to transfer a culture aseptically. The loop must always be flamed again after use

Selective media contain substances which selectively inhibit the growth of certain microorganisms, whilst allowing others to grow. These media are particularly important in medical microbiology, as they are used to culture and isolate organisms from clinical specimens such as blood arid urine. An example of a selective medium is MacConkey agar, which contains lactose and bile salts. This is used to isolate enteric bacteria, that is, bacteria which grow in the intestinal tract. Bacteria such as Escherichia coli, which are able to utilise

lactose as an energy source, are able to grow, but the growth of other species of bacteria, including Staphylococcus spp. will be inhibited.

Figure:3 Using a bacteriological loop to carry out an aseptic transfer

Some microbiological media contain a coloured pH indicator substance, such as phenol red or bromocresol purple. These are known as indicator media (or differential media) and will show whether or not a change in pH has occurred as a result of the metabolism of the bacteria during growth. For example, if the bacteria produce acids, then a broth medium containing phenol red will change in colour from red to yellow. Eosin-methylene blue (EMB) agar is an example of a medium which is both selective and an indicator medium and is used to isolate Gram-negative enteric bacteria. EMB agar contains lactose and sucrose as energy sources, and the dyes eosin and methylene blue. Methylene blue inhibits the growth of. Gram-positive bacteria; eosin changes colour according to the pH of the medium,

changing from colourless to black in acidic conditions. *E. coli* will grow in EMB agar to produce colonies which are black, with a greenish metallic sheen. Salmonella produces colonies which are translucent, or pink.

Although selective media are important in the isolation and identification of bacteria, many species can be successfully cultivated using nutrient broth, or nutrient agar. Broth media may be solidified by the addition of agar, a polysaccharide obtained from red algae. Agar is usually added at about 1.5 per cent by weight to the medium, and dissolved by boiling. On cooling to about 42 °C, the medium will set to produce a clear, firm gel.

Use of fermenters

Microorganisms may be grown on a large scale for the purposes of producing a wide range of useful products including antibiotics, enzymes, food additives and ethanol.

Fermenters are vessels used for the growth of microorganisms in liquid media. These vary in size from small scale laboratory fermenters containing perhaps 250 cm^3 of medium, to very large scale industrial fermenters containing up to 500 000 dm^3. The majority of microorganisms grown are aerobic and it is therefore essential to ensure an adequate supply of oxygen to maintain aerobic conditions.

Two main systems for culturing microorganisms are used, referred to as batch culture and continuous culture. In batch culture, growth of the microorganism occurs in a fixed volume of medium and, apart from oxygen, substances are not normally added to the medium during culture. The organism typically goes through the usual phases of growth, that is, lag, exponential and stationary. The organism continues to grow in the medium until conditions become unfavourable. In continuous culture, fresh, sterile medium is added to the fermenter at a constant rate and spent medium, together with cells, is removed at the same rate. The number of cells and the composition of the medium in the fermenter therefore

remains constant. Continuous culture can, theoretically, run indefinitely but, apart from the production of Quorn™ mycoprotein, few industrial cultures are maintained continuously.

To illustrate the principle of a fermenter, Figure:4 shows a simple fermenter which is suitable for use in a school laboratory.

This fermenter could be used to grow an organism such as yeast (*Saccbaromyces cerevisiae*) under controlled conditions. Before use , the