قسم التقنيات الاحيائية

المادة : احياء مجهرية بيئية

المرحلة الثانية

الكورس الاول

م .م طالب صالح قادر

م .م مروة شاكر

م. هبة عبد الرحمن

Environmental Microbiology/ Lab Lectures

Lab No: 1

Introduction

The purpose of this laboratory work is to guide students successfully to completing the practical side of course will demonstrate the ability to:

1- Use standard laboratory equipment correctly, including using the standard metric system form weights, lengths, diameters, and volumes, using an incubator.

2- Correctly Use, setting up, focusing, handling & cleaning a bright-field light microscope.

3- Properly use aseptic techniques autoclaving, sterilizing and maintaining sterility.

4- Designing of a successful sampling strategy.

5- Estimate the number of microorganisms in a sample using serial dilution techniques, including: correctly spreading, diluted samples for counting & estimating appropriate dilutions.

6- Communications and Interpersonal skills, including discussing and presenting laboratory results, working effectively in groups or teams.

Introduction to Microbiology

Microorganisms, living creatures too small to be seen with the unaided eye, have a profound Influence on daily life - often beneficial, sometimes harmful. Like bacteria, fungi, algae, protozoa

Bread is made with the help of yeast that ferment sugar and produce carbon dioxide in the dough before it is baked.

Cheese, yogurt and sour cream require the presence of microorganisms that sour the milk from which they are made.

Antibiotics cure such bacterial diseases as “step” turret bacterial pneumonias, gonorrhea and syphilis.

LAB2\Dilution and Plating of Bacteria and Growth Curve Introduction

Perhaps the most widely used technique for the study of bacteria is the growth of a microbe of interest in a liquid nutrient medium, followed by dilution and plating on a solid agar medium. Here the theory is that one colony arises from one organism. Each colony is then referred to as a colony forming unit (CFU). In addition to providing an estimate of bacterial numbers, this procedure allows the opportunity to obtain pure culture isolates. Often times, researchers will measure the turbidity of the liquid culture at different time intervals using a spectrophotometer. The comparison of turbidity with plating results allows for a quick estimation of bacteria numbers in future studies. These techniques are used in all aspects of microbiology including clinical and environmental microbiology. The growth of a bacterial isolate will be followed as a function of time to illustrate the various phases of growth that occur in liquid culture. Intuitively one can recognize that bacterial growth (via cell division) in liquid media will continue to occur until: a) nutrients become limiting; or b) microbial waste products accumulate and inhibit growth. To understand and define the growth of a particular microorganism, cells are placed in a flask in which the nutrient supply and environmental conditions are controlled. If the liquid medium supplies all the nutrients required for growth and

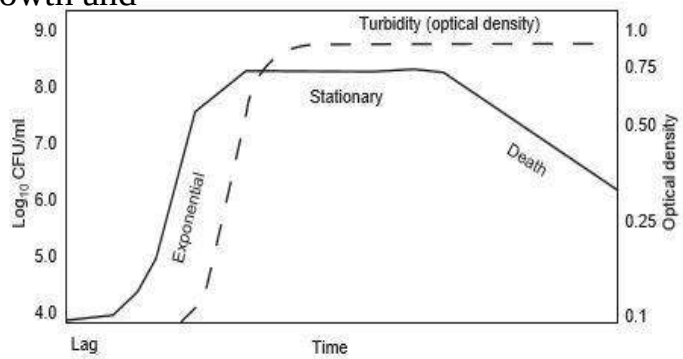

Figure – 1: A typical growth curve for a bacterial population. Compare the difference in the shape of the curves in the death phase (colony-forming units (CFUs) versus optical density). The difference is due to the fact that dead cells still result in turbidity.

environmental parameters are conducive to growth, the increase in

LAB3\ Preparation of Microbiological Culture Media

Culture media are the nutrient solutions used in laboratories to grow microorganisms. For the successful culture of a given microorganism it is necessary to understand its nutritional requirements and then supply it with its essential nutrients in the proper form and proportions in a culture medium. The general composition of a medium is as follows:

C-source, N-source, Inorganic nutrients e.g. S, P, Trace elements (Zn, CO, Cu, Mn, Mg, Pb….etc), Growth factors (aminoacids, purines, pyrimidines and vitamins), Solidifying agent (e.g agar), Solvent (usually distilled water).

According to the consistency three types of media are used: liquid, or broth, media; semisolid media; and solid media.

The major difference among them is that solid and semisolid media contain a solidifying or gelling agent [such as agar, gelatine], whereas a liquid medium does not.

- Liquid media, such as nutrient broth, tryptic soy broth or glucose broth can be used in studies of growth and metabolism in which it is necessary to have homogenous media conditions, to follow optical density, and to allow early sampling for analysis of substrates and nd metabolic products. Tubes and flasks with liquid cultures can be incubated with either static or shaken incubation.
- Semisolid media can also be used in fermentation studies, in determining bacterial motility, and in promoting anaerobic growth.
- Solid media, such as nutrient agar, are used for: 1) The surface growth of microorganisms in order to observe colony morphology, 2) For pure culture isolation, 3) Often in the enumeration and isolation of bacteria from a mixed population by diluting the original bacteria suspension and spreading a small inoculum over the surface of the solidified medium. 4) To observe specific biochemical reactions (extracellular enzymes diffusing away from the colony can be detected as a result of their action on insoluble substrates present in the agar medium).

Solid media can be poured into either a test tube or Petri dish. If the medium in the test tube is allowed to harden in a slanted position, the tube is designated an agar slant; if the tube is allowed to harden in an upright position, the tube is designated an agar deep tube; and if the agar is poured into a Petri dish, the plate is designated an agar plate

LAB4

Bacteriological Examination of Water: The Coliform MPN Test

Introduction

Microorganisms pathogenic to the humans that are transmitted by water include: bacteria, viruses, and protozoa. Most of the microorganisms transmitted by water usually grow in the intestinal tract of man and leave the body in the feces.

Fecal pollution of water used for swimming and drinking can then occur resulting in transmission of infectious microorganisms.

Coliform bacteria (of which *Escherichia coli* is a member) are often associated with enteric pathogenic organisms and have been shown to be useful indicators of the presence of fecal contamination.

When members of the coliform group are present, other kinds of microorganisms capable of causing disease also may be present.

The coliform group includes all aerobic and facultative anaerobic, Gram negative, non-spore-forming, rod-shaped bacteria which ferment lactose with gas production in prescribed culture media within 48 hours at 35°C. Coliform bacteria include *Escherichia coli*, *Citrobacter*, *Enterobacter*, and *Klebsiella* species.

An MPN test and the membrane filter test have been the methods most commonly used for the detection of coliforms in water.

The (Most Probable Number) **MPN** test for coliforms consists of three steps: a test, a **Confirmation** test, and a **Completed** test.

The first step is the presumptive test. A set of tubes of MacConkey broth is inoculated with samples of water and incubated. MacConkey broth contain Bile salt which inhibits the growth of gram-positive organisms while encouraging the growth of coliforms. Coliforms use any oxygen present in the broth and then ferment the lactose producing acid and gas under anaerobic conditions. Gas formation in 24 or 48 hours is a positive test

Lab No: 5

Isolation of fungi and Actinomycetes from soil

1. **Fungi**

Fungi comprise a large group of eukaryotic non-photosynthetic organisms (have no chlorophyll) that include two forms yeasts and molds. They are widely distributed in the environment, including soil, air, water, plants and animals.

Fungi may be saprophytic or parasitic organisms, their presence associated with organic matter, therefore, they may consider as good *pollution indicators*; they are either unicellular or multicellular (filamentous), lack tissue differentiation, have cell walls of chitin or other polysaccharides, and propagate by spores.

The importance of fungi in the environment referred to the following:

Their pathogenesis to human, animals and plants.

Their role as source of food for human and animals.

They are vital decomposers in the ecosystem, breaking down dead organisms and biological waste, freeing nutrients for use by other organisms and clearing away their remains.

They naturally produce the antibiotics, which kill or inhibit the growth of pathogenic microorganisms.

Isolation procedure

a- Collect soil samples from cultivated and uncultivated soils from different locations.
b- place them in sterile container.
c- Prepare soil solution by adding 5gm of a soil sample to 50ml sterile distilled water and shaking for 10 minutes.

LAB6 \ **Water Quality Standards & Isolation of Some Water - Borne Pathogens**

Introduction:

Water Quality: The degree to which water is pure enough to fulfill the requirements of various uses. Any physical, chemical, or biological change in water quality that adversely affects living organisms or make water unsuitable for desired uses can be considered pollution.

Microorganisms are often associated with higher organisms and in most cases this association is of significance to both the host and the microbe. In fact, mounting evidence indicates that microbes are important to their host's overall health. On the other hand, clearly some microbes can be detrimental to their hosts. Indeed, there are hundreds of pathogenic microorganisms responsible for millions of deaths and illnesses. Many human pathogens of concern are associated with feces, and therefore fecal contamination is an important source of illness in both developed and developing countries (Table 1). Water is an important means for the transmission of pathogens. Although the importance of fecal pollution of water in the transmission of disease. Diseases such as cholera, typhoid fever, bacterial & amoebic dysentery, hepatitis, malaria, yellow fever & other diseases were found to be associated with sanitary conditions of water, and as a result, management practices preventing the pollution of water were subsequently implemented.

(Table 1): Pathogens Carried by Sewage and their Production Diseases

Disease	Infectious agent
Typhoid fever	*Salmonella typhi*
cholera	*Vibrio cholera*
diarrhea	*Escherichia coli*
Infectious hepatitis	Hepatitis A virus
dysentery	*Shigella* spp. (bacteria), *entamoeba histolytica* (protozoan)

LAB7\ **Biological Oxygen Demand Measurement (BOD)**

Biological Oxygen Demand (BOD) is the amount of dissolved oxygen needed by aerobic microorganisms to break down organic pollutants present in the sample, under specified condition(temperature 20 °C and darkness), within a certain period of time (after 5 days).

Importance:

BOD is an important water quality parameter because it provides an index to assess the effect discharged wastewater will have on the receiving environment. The higher the BOD value, the greater the amount of organic matter or "food" available for oxygen consuming bacteria.

BOD measurement can be used to evaluate the impact of biodegradable substances in water & waste by measuring the quality of water & treatment result in waste water. Depending on the measurement site and type waste water the BOD value can lie between a few mg/ L & several thousand mg/ L.

The BOD serves as a bulk parameter indicating:

1- Level of organic pollution of the waste water.
2- The quantity of oxygen needed for the respiration of the organism contain in the BOD bottle during the incubation period.
3- Evolution the efficiency of the treatment system through the reduction in BOD value.

The BOD is made up from tow reaction during 5 days

1- Oxygen demand for endogenous cellular respiration.
2- Oxygen demand for decomposition & metabolism of carbon & hydrogen compounds.

Collection of sample

Collect sample very carefully, do not let the sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth is steam lakes & or reservoirs, need special precautions to eliminate changes in pressure and temperature.

Collect entraining or dissolving atmospheric oxygen

LAB8\Antibacterial activity of bioactive compounds produced by *Streptomyces* spp. isolated from agricultural soil

Introduction:

Streptomyces sp. is Gram-positive bacteria. These bacteria were first regarded as fungi because of the superficial similarity in the filaments between them and fungi. However, then, they will classify as true bacteria. *Streptomyces* sp. is present in a wide range of environments, either as dormant spores or actively growing. The common habitat of this bacteria is soils. Among the microorganisms, *Streptomyces* which belonging to the Actinomycetes group gained special importance in medical and biotechnology industries due to their ability to produce a vast number of bioactive molecules. They are the most important producers of bioactive secondary metabolites. They produce vitamins, enzymes, antitumor agents, anti-cancer agents and mainly antibiotic compounds. In fact, most antibiotics in clinical use are direct natural products or semisynthetic derivatives from actinomycetes and fungi. Approximately 7000 of the compounds (antibiotics) reported in the Dictionary of Natural Products were produced by Actinomycetes. Almost 80% of bioactive compounds are derived from Actinomycete metabolites, mostly from the genus *Streptomyces.*

The similarities between *Streptomyces* and fungi:

Production of hyphae and conidia

Non- logarithmic cell division as the case in bacteria

Production of flocculent during growth inside liquid medium in contrast to bacteria which produce turbidity.

The similarities between *Streptomyces* and bacteria:

Both are prokaryotes, while fungi are eukaryotes.

Cell wall consists of peptidoglycan; in fungi it consist of cellulose and chitin.

Both are sensitive to antimicrobial agents.

Their cell diameters are close to each other, ab

LAB9\Effect of environmental factors on microbial growth

Chemical factors	Form usually found in nature	Chemical form commoly added media
carbon	CO_2, organic compounds	Organic; simple sugars e.g. glucose, acetate or pyruvate; extracts such as peptone, tryptone, yeast extract etc.
oxygen	Water (H_2O), organic compounds	
hydrogen	Water (H_2O) organic compounds	
nitrogen	NH_3, amino acids	Organic; amino acids,nitrogenous bases. Inorganic; NH_4CI
phosphorus	PO^4	KH_2PO_4, Na_2HPO_4
potassium	K^+	KCI, K_2HPO_4
Magnesium	Mg^{2+}	$MgCI_2$, $MgSO_4$
Calcium	Ca^{2+}	$CaCI_2$
Sodium	Na^+	NaCI
Iron	Fe^{3+} organic iron complexes	$FeCI_3$
Trace elements	Usually present at very low concentrations	$ZnCI_2$, $CuCI_2$
Organic growth factors	Usually present at very low concentrations	Vitamins, amino acids, purines, pyrimidines

determined the different factors and conditions that can affect microbial growth. As environmental conditions are constantly changing, the growth of a microbe can change with it. A shift in the condition can either inhibit or slow the rate of growth. The growth of a microbe is a function of various activities, from nutrient transport to the synthesis of molecules. Each activity has a certain enzymes that help progress or inhibit each step, and each enzyme has an optimum condition that it works best in. As conditions change, the ability of enzymes to perform the basic functions of the microbe was affected. Knowing the conditions required for growth of each organism can help biologist to determine where certain microbe can be found or help to inhibit growth and stop microbes from spreading.

Growth of microorganisms is greatly influenced by a number of environmental factors such as type of nutrient materials, oxygen requirement, temperature, pH, osmotic pressure and radiation. Microbial growth requires suitable environmental conditions, a source of energy, and nourishment. These requirements can be divided into two categories, physical and chemical.

Lab 10/The Microorganisms Found in Sewage

Microorganisms found in sewage originate from two sources--soil and sanitary waste. One milliliter of sewage typically contains between 100,000 and 1 million microorganisms, according to the Mountain Empire Community College website. While most of these organisms, such as various types of bacteria, play a pivotal role in the decomposition of waste and are considered an integral component of organic matter, some are pathogenic, or disease-carrying, and pose a threat to public heath.

Parasitic Bacteria

Bacteria are single-cell organisms that proliferate in suspended matter, such as sludge. When they encounter a supply of nutrients, they feed by taking in food directly through the cell wall and reproduce quickly. Among the numerous types of bacteria in sewage, the most common types are fecal coliforms, which originate in human intestines and travel via human discharges. These parasitic bacteria require a living organism, or host, and a readily available food supply.

Pathogenic Bacteria

Specific forms of parasitic bacteria manufacture toxins that cause disease in the host organism. These pathogenic types of bacteria may be discharged by people who suffer from dysentery, cholera, typhoid fever and other diseases of the intestines. Pathogens typically found in sewage include Salmonella, Shigella, E. coli, Streptococcus, Pseudomonas aeroginosa, mycobacterium and Giardia Lamblia, according to the Water Quality and Health Council website. Shigellosis outbreaks have resulted from freshwater shellfish contaminated by wastewater flows, as chronicled by the U.S. Centers for Disease Control and Prevention. Due to population growth and increased discharge into wastewater, the resulting abundance of pathogenic bacteria overwhelms natural processes of decomposition and dilution.

Saprophytic Bacteria

Saprophytic bacteria devour dead organic substances, which helps to break down waste into inorganic and organic byproducts. These bacteria play a critical role in the treatment of sewage by facilitating or accelerating the decomposition process of organic matter. Without saprophytic bacteria decomposition may not occur. The different species of saprophytic bacteria perish after having played their part in the relevant stage of decomposition.

Viruses

Among the microbes found in sewage are viruses, which are parasitical organisms that require living matter to feed on, grow and reproduce. Pathogenic viruses that exist in wastewater include polio and hepatitis. Various intestinal viruses, such as coxsackie, adenovirus and ECHO, or enteric cytopathic human orphan, are also found