قسم التقنيات الاحيائية

مادة : تقنيات حيوية بيئية

المرحلة الثالثة

الكورس الثاني

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Lab 1/

Introduction

Production of cellulase by microorganisms

Cellulose is one of the main components of plant cell walls. It consists of long chains of glucose molecules. These can be broken down by a number of fungi and bacteria found in soil and by certain bacteria in the rumen (the grass-digesting part of the gut) in cows and other ruminants. These microbes produce cellulase enzymes that can degrade certain types of cellulose outside the cell into products which include glucose. Cellulolytic bacteria include species of *Cellulomonas*, *Pseudomonas* and *Ruminococcu*s. Cellulolytic fungi include *Chaetomium*, *Fusarium*, *Myrothecium* and *Trichoderma*. You are going to investigate the effectiveness of enzymes in breaking down different types of papers.

A large part of paper is the chemical substance called cellulose. Paper is made from woody plants and cellulose makes up 40-50% of the cell walls of plants. The molecules are very large and very long – so they are not soluble in water. But if they are digested or broken down by enzymes from microbes, they are changed into smaller molecules that will dissolve in water. Once the cellulose is broken down, microbes (and other living things) can use it as nutrient. In this investigation you will find out how microbes in the soil change paper over a few weeks. You might be able to find out about paper in our rubbish and cellulose in composting as part of this investigation.

The aims of this practical are:

- To investigate how quickly different kinds of paper decompose under the action of soil microbes
- To explore the role of soil microbes in the carbon cycle
- To introduce the use of enzymes in industrial processes

Procedure in the lab

1. Label 6 test tubes A-F, and add the date.
2. Use the graduated pipette and filler to place 5 cm^3 of nutrient broth in a test tube. Carefully drop in a 1 cm x 2 cm sample of filter paper.

LAB2\

Bioremediation is the use of microbes to clean up contaminated soil and groundwater, most commonly referred to as a microbial process. The population of microbes may use the soil matrix, groundwater or both as a substrate. The soil is amended with nutrients, while moisture and oxygen are maintained at near optimum levels for degradation to occur. The aerobic microbes utilize the organic carbon in the contaminant as an energy and organic carbon source for growth. They typically utilize oxygen and produce carbon dioxide and water in this process.

There are two basic strategies use for microbial based bioremediation system; ***in situ*** and ***ex situ*** bioremediations. Selection of the appropriate technology depends on the <u>solubility</u>, <u>volatility</u> and <u>adsorptive ability of the contaminant</u>, and the <u>location and the extent of contamination</u>.
In-situ **techniques:** are most effective on soluble, dissolved, and volatile contaminants. It has used primarily at sites contaminated with light petroleum derivatives such as gasoline and diesel fuel and contaminants are destroyed with a minimum of disruption to the surface environment.
Ex-situ **system:** treat the soils or groundwater after removal from it original environment. ***Ex-situ*** treatment techniques are classified as land treatment, compositing, or slurry phase. Soils contaminated with highly sorbet contaminants such as heavy fuel, oil sludge and pesticides have been effectively treated.

<u>Factors affecting microbial bioremediation:</u>
The rate of microbial remedation of organic pollutants is dependent upon many biological, chemical and physical parameters. Environmental enhancements, such as adjustment of pH, temperature, nutrient level, and aeration, are necessary to facilitate the bio-transformation process.

LAB3

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.

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The BOD is made up from tow reaction during 5 days

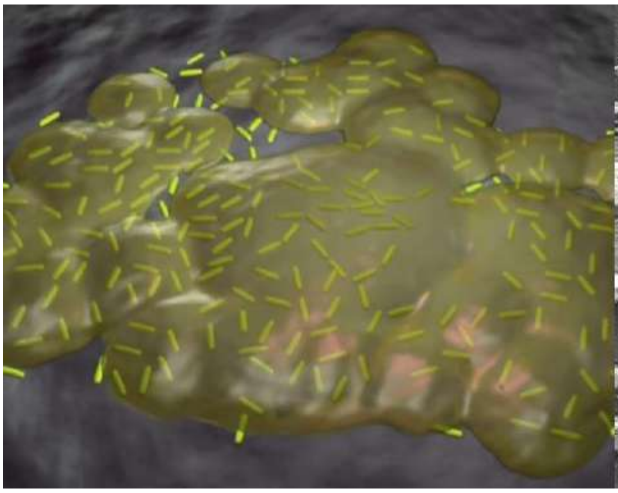
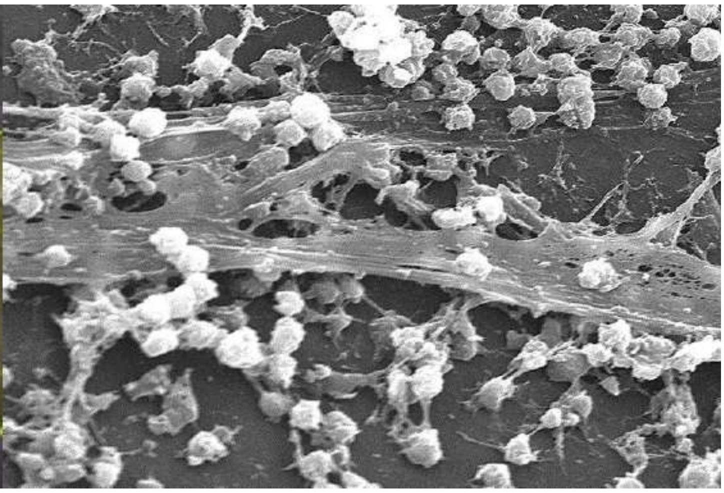
1- Oxygen demand for endogenous cellular respiration.

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.

LAB4**Biofilm**

A biofilm is any group of microorganisms in which cells stick to each other and often these cells adhere to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. While still not fully understood, it is thought that the first colonists of a biofilm adhere to the surface initially through weak, reversible adhesion via van der Waals forces and hydrophobic effects. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.

There are five stages of biofilm development:

1. *Initial attachment*.
2. *Irreversible attachment* (cell-cell adhesion).
3. *Proliferation.*
4. *Maturation*.
5. *Dispersion*.

LAB5\ **Biodegradation of Phenol compounds**

Due to rapid industrialization and economic development, many pharmaceutical and chemical industries are releasing their effluents into the natural ecosystem and they are inhibiting the sunlight penetration and reducing the photosynthetic activity of aquatic system as well as these wastes have a harmful (toxic) effect on living organisms. Among all these chemical pollutants, phenol is the most toxic compound.

Phenol is a naturally occurring compound in the environment and is readily biodegradable. Phenol compounds are introduced into the environment through the waste water stream from several industrial operations, through its use as antimicrobial agent or as by-product of other pharmaceutical industries, It is used as a general disinfectant, in the manufacture of colorless or light-colored artificial resins, many medical and industrial organic compounds, dyes and solvents and it is used as a reagent in chemical analyses and also used in insecticides structure.

The hazardous effects of phenol usually came from its ability to persist in the environment for a long time due to its high stability, bioaccumulation in human and animal tissue.

There are different methods available for phenol removal such as solvent extraction, adsorption, chemical oxidation. All these non-biological methods have serious drawbacks such as high cost and formation of hazardous byproducts, whereas biological degradation (biodegradation) is generally preferred due to lower costs and the possibility of complete mineralization.

Biodegradation is a useful strategy to eliminate organic compounds and detoxify wastewaters and polluted environments. Phenol is degraded by diverse microorganisms including yeasts, molds and bacteria. Because of widespread occurrence of phenol in the environment, many microorganisms utilize phenol as the sole carbon and energy source for their growth and reproduction, which includes both aerobic and anaerobic microorganisms such as *Staphylocoocus, Bacillus, Pseudomonas, Aspergillus and Candida*.

LAB6\

Production of biosurfactant by bacteria

Biosurfactants are surface-active substances derived from living organisms, especially microorganisms.

Biosurfactants are amphiphilic compounds, containing hydrophobic and hydrophilic moieties. The hydrophilic moiety can be carbohydrate, amino acid, phosphate group or some other compounds, whereas the hydrophobic moiety usually is a long chain fatty acid.

Biosurfactants are being investigated as replacements for synthetic surfactants **because** they are biodegradable, less sensitive to extreme environments and can be produced on renewable substrates.

The potential applications of biosurfactants in industrial include emulsification and foaming for food processing, wetting and phase dispersion for cosmetics and textiles, or solubilization for agrochemicals and in pharmaceutical products. In addition, biosurfactants can be used in environmental applications such as bioremediation and dispersion of oil spills as well as solubilize different chemical pollutants.

Biosurfactants can be divided into **4 groups** based on their overall structures. They are glycolipids, phospholipids, lipoproteins or lipopeptides.

Biosurfactants can be commercially produced at levels of up to 100 g/L, as reported for rhamnolipids from *Pseudomonas sp.* This production level, combined with the use of cheap renewable substrates as organic wastes, makes the cost of biosurfactants competitive with the cost of synthetic surfactants.

Alternative substrates have been suggested for biosurfactant production, especially water-miscible agro-industrial

LAB7\

Bioabsorption of heavy metal by microorganism

Industrialization has led to the introduction of heavy metals in the environment. Heavy metals are known to persist in the environment and become a risk for organisms. Microorganisms are present in industrial effluents. They have adopted different strategies to cope up with the harmful effects of these metals. These strategies can be metabolism dependent or independent. One such strategy is bioabsorption, which is binding of metal ions with metal binding proteins present in the cell wall.

-**Heavy Metals** are chemical elements with a specific density usually more than $5g/cm^3$, which is five times higher than water.

- **The heavy pollutants** include: lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel.

-**Heavy metals** become toxic when they are not metabolized by the body and accumulate in the soft tissues.

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physical such as(Ion exchange and filtration), chemical methods

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:

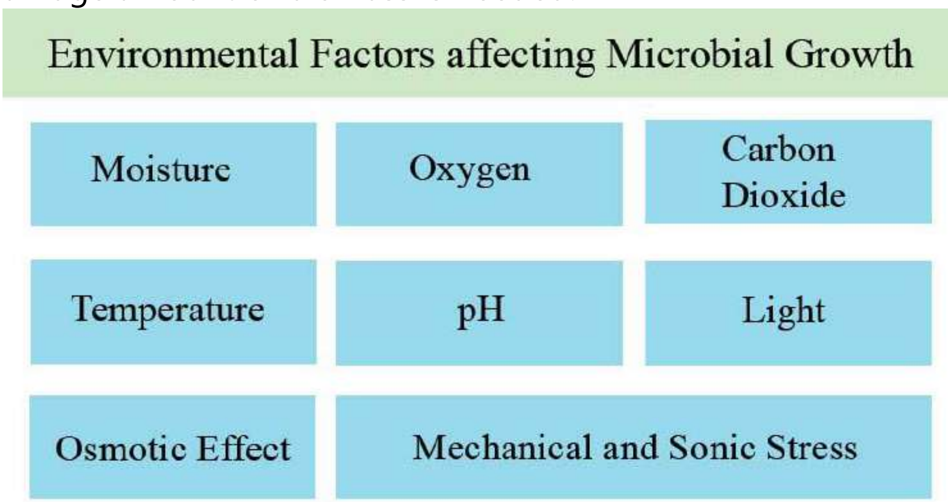
- Both are prokaryotes, while fungi are eukaryotes.
- Cell wall consists of peptidoglycan; in fungi it consist of cellulose and chitin.
- Both are sensitive to antimicrobial agents.
- Their cell diameters are close to each other, about 0.1- 1µm.

The term "bioactive" is composed of two words: bio which means life and

Lab 9

Environmental Factors affecting Microbial Growth

Apart from nutritional components growth of the microbes are also dependent on several environmental factors. These factors play an important role in understanding the growth pattern of a microbe. Apart from that, it is important to understand these factors especially in an industrial set up where a huge amount of biomass is needed.



The environmental factors include:

1. Moisture

Water is an essential component for the growth of the bacteria. 80% of the bacterial cell is made up of water. Therefore, the presence of a free water molecule is important for the optimum growth of the microorganism. Apart from that desiccation or drying has a severe effect on microbes. For example *Treponema pallidum, N. gonorrhoeae* can die easily due to desiccation. While bacterial pathogens like *M. tuberculosis, S. aureus* can survive desiccation for several weeks. Therefore, it is understood the required moisture content buys the microbe varies species to species. Hence, the moisture content needs to be maintained for optimum growth.

Lab10 / bioconversion (biotransformation

Biotransformations are structural modifications in a chemical compound by organisms /enzyme systems that lead to the formation of molecules with relatively greater polarity

This mechanism has been developed by microbes to acclimatize to environmental changes and it is useful in a wide range of biotechnological processes

The most significant aspect of biotransformation is that it maintains the original carbon skeleton after obtaining the products

Biotransformation is of two types: Enzymatic and Non-enzymatic. Enzymatic are further divided into Microsomal and Non-microsomal.

Enzymatic Elimination is the biotransformation occurring due to various enzymes present in the body. Microsomal biotransformation is caused by enzymes present within the lipophilic membranes of smooth endoplasmic reticulum.

Non-Microsomal Biotransformation involves the enzymes which are present within the mitochondria.

Examples include: Alcohol dehydrogenase responsible for metabolism of ethanol into acetaldehyde and Tyrosine hydrolases enzymes, Xanthine oxidase converting hypoxanthine into xanthine etc.

Spontaneous, non-catalyzed and non-enzymatic types of biotransformation are for highly active, unstable compounds taking place at physiological pH.

Some of these include Chlorazepate converted into Desmethyl diazepam, Mustin HCl converted into Ethyleneimonium, Atracurium converted into Laudanosine and Quartenary acid, Hexamine converted into Formaldehyde.

Microbial biotransformation is widely used in the transformation of various pollutants or a large variety of compounds including hydrocarbons, pharmaceutical substances and metals.[7] These transformations can be congregated under the categories: oxidation, reduction, hydrolysis, isomerisation, condensation, formation of new carbon bonds, and introduction of functional groups