قسم التقنيات الاحيائية

المادة : تقنيات التخمرات

المرحلة الثالثة

الكورس الاول

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Fermentation technology Lab. 1

Isolation of Industrial Microorganisms from Soil and their Potential to Produce Antibiotics

Introduction

Microorganisms are used extensively to provide a vast range of products and services. They have proved to be particularly useful because of:

1- The ease of their mass cultivation

2- Speed of growth

3- Use of cheap substrates (which in many cases are wastes)

4- The diversity of potential products.

5- Their ability to readily undergo genetic manipulation.

The major sources of microbial cultures for use in industrial microbiology were natural materials such as soil and waters. There are few hundred species of microorganisms that are used to make useful products. These are called **industrial microorganisms**, they include:

1- Bacteria

2- Actinomycetes (filamentous bacteria such as *Streptomyces*)

3- Molds (filamentous fungi): *Penicillium chrysogenum, Penicillium notatum, Aspergillus niger, Aspergillus oryzae*

4- Yeasts : *Saccharomyces cerevisiae , Candida utilis*

The microorganisms employed by industry have been isolated from nature, and in many cases, were modified using classic mutation-selection procedures. Genetic engineering has replaced this more traditional approach to developing microbial strains of industrial importance.

The first task for an industrial microbiologist is to find a suitable microorganism for use in the desired process and has the following characteristics.

(a) Genetically stable

(b) Easy to maintain and grow

Fermentation Technology Lab. 2

Production of ethanol by yeast

Introduction

Many chemical compounds are prepared by the action of microorganisms, acting on appropriate starting materials. One of the oldest of such processes is the preparation of ethyl alcohol (ethanol) from carbohydrates (sugars) by fermentation. Ethanol production by yeast (ethanol fermentation) is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, ethanol fermentation is classified as anaerobic

For this experiment, the microorganism is yeast which will convert glucose to ethanol by fermentation according the following chemical reaction:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

As the alcohol concentration increases, the yeast suffers inhibition and ultimately death. The maximum concentration of alcohol in water that most yeast can tolerate is about 12%.

There are many environmental conditions that affect yeast cell growth and the kinetics of chemical reactions within living cells. These include the availability of major and minor nutrients, the temperature, pH, and dissolved oxygen concentration.

The amount of dissolved oxygen in the fermentation broth has major implications for the reactions that occur in yeast. **When oxygen is present**, respiration occurs, converting simple sugars to biomass and carbon dioxide. **In the absence of oxygen**, yeast switch to alcoholic fermentation.

The purpose of the laboratory:

The purpose of experiment is to produce ethanol by *Saccharomyces cerevisiae* using dates juice as a substrate.

Fermentation technology Lab. 3

The rate of fermentation varies with the type of sugar being metabolized.

❖ Introduction

Many yeasts of the genus *Saccharomyces* are facultative anaerobes. This means that, in the presence of oxygen, they will oxidize a carbon source, such as glucose. Carbon dioxide and water are produced. On the other hand they can grow in the absence of oxygen. When this happens a form of respiration occurs in which the sugar is converted to ethanol and carbon dioxide. This conversion is usually called *fermentation.* It is brought about by a sequence of more than fourteen chemical reactions in a *metabolic pathway.*

The rate at which carbon dioxide is produced can be used as a measure of the overall rate of fermentation. This investigation examines how the rate of fermentation varies with temperature and with the type of sugar being metabolized.

❖ The purpose of the laboratory

The purpose of this experiment is to exam how the rate of fermentation varies with the type of sugar being metabolized

❖ *Procedure*

1- Prepare a yeast suspension by stirring 4g of dried yeast and 1 g of yeast extract into 100ml of water.

2- Start a yeast culture by thoroughly mixing 20ml of the yeast suspension with 20ml of glucose solution (0.2molmdm~ 3). (This volume should be sufficient for twelve experiments.) Incubate this in a water bath at 35 °C for at least one hour to let fermentation start.

3- Repeat step 2 with any other available fermentation substrates. These might include sugars such as galactose, which is very similar to glucose, and fructose, lactose, and sucrose. *Include a suitable control.*

4- Place two clean test-tubes next to each yeast culture tube in the water bath. Transfer 2-3 ml of the culture into each test-tube.

5- Next, a small Durham tube has to be filled with yeast culture and inverted in each test tube. To do this, follow the instructions *in figure 1*

Fermentation technology Lab. 4

How fermentation varies with changes in temperature

❖ Introduction

Many yeasts of the genus *Saccharomyces* are facultative anaerobes. This means that, in the presence of oxygen, they will oxidize a carbon source, such as glucose. Carbon dioxide and water are produced. On the other hand they can grow in the absence of oxygen. When this happens a form of respiration occurs in which the sugar is converted to ethanol and carbon dioxide. This conversion is usually called *fermentation.* It is brought about by a sequence of more than fourteen chemical reactions in a *metabolic pathway.*

The rate at which carbon dioxide is produced can be used as a measure of the overall rate of fermentation. This investigation examines how the rate of fermentation varies with temperature.

❖ The purpose of this laboratory:

The purpose of the experiment is to exam how fermentation varies with changes in temperature

❖ Procedure

1- Set up three water baths at temperatures between 20 °C and 50 °C *(e.g.* 20 °C, 35 °C, and 50 °C). Set up a culture of yeast at 35 °C containing: 2g dried yeast, 3g glucose, 1 g yeast extract, 100ml water. Incubate for at least one hour to allow fermentation to start.

2- Transfer 2-3 ml of the culture to each of six test-tubes. Place two of these in each water bath. Allow them to equilibrate for five minutes.

3- Next, a small Durham tube has to be filled with yeast culture and inverted in each test tube.

4- Follow the instructions *in figure 1* to fill Durham tubes and insert them upside down into each test-tube. (Do not remove the test-tubes from their water baths for too long.)

Fermentation technology Lab. 5

Fermentation of lactose by lactic acid producing bacteria: Yoghurt

❖ Introduction

Two bacteria are involved in making yoghurt: *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Both are homolactic (homofermentative); that is, they use lactose as an energy source and produce lactic acid as their sole fermentation product. Initially, the *Streptococcus thermophilus* is dominant, then it is inhibited by the produced acid. In a second phase, the
Lactobacillus bulgaricus continues to ferment the remaining lactose; the pH drops from 6.5 to about 4.5.

 Description of the *Streptococcus thermophilus*

Gram positive spherical or ovoid cells, 0.7-0.9 µm in diameter in pairs t o long chains. Final pH range in glucose broth is 4.0-4.5. The preferential fermentation of the disaccharides, sucrose and lactose, may result in a lower pH value as compared to glucose fermentation. Acid is produced from glucose, fructose, lactose and sucrose; no acid from trehalose, maltose, inulin, glycerol, mannitol, sorbitol or salicin and rarely from raffinose, xylose or arabinose. Optimum temperature is between 40°C and 45°C. Growth occurs at 50°C but not at 53°C. No growth at temperatures below 20°C. Heat tolerance: survives 65°C for 30 min. Source: milk and milk products such as cheese and yoghurt. Often used as a starter culture for these products. This species is easily recognized by its thermal tolerance; unable to ferment maltose and unable to grow in media containing ≥2.0% sodium chloride.

 Description of *Lactobacillus bulgaricus*

Gram positive rod, width <1 µm, contains aldolase, is negative for catalase, indole, nitrate reductase, oxidase, and benzidine reactions, attacks glucose and produces lactic acid as the major product. Does not ferment adonitol, dulcitol, erythritol, glycerol, glycogen, inositol, inulin, sorbose, starch. Does not produce gas from ribose, gluconate or glucose. Requires niacin, riboflavine and pantothenate. Produces up to 1.7% acid in milk, does not produce NH3 from arginine. Utilizes lactose, and weakly also fructose, galactose, mannose. Does not utilize aesculin, amygdalin, arabinose, cellobiose, maltose, mannitol, melezitose, melibiose, raffinose,

Fermentation Technology Lab. 6

Yeast fermentation with and without aeration

❖ Introduction

Yeasts are single-celled fungi that can derive energy from sugar molecules. They can also break down larger carbohydrate molecules (like starches present in flour) into simple sugar molecules, which are then processed further.

Yeast can obtain more energy from sugar when oxygen is present in their environment. In the absence of oxygen, yeast switch to a process called *fermentation*. With fermentation, yeast can still get energy from sugar, but less energy is derived from each sugar molecule

In addition to deriving less energy with fermentation, the end products of sugar metabolism are also different. When oxygen is present, the sugar molecules are broken down into carbon dioxide and water (plus the energy that the yeast uses to grow and reproduce). In the absence of oxygen, the fermentation process produces alcohol, carbon dioxide and water (and less energy).

In this experiment, you will grow yeast in containers with and without aeration, and compare the amount of carbon dioxide in the two conditions.

❖ Objective

The objective of this experiment is to investigate yeast metabolism under aerobic and anaerobic conditions by measuring carbon dioxide output

❖ Materials and Equipment

For this project you will need the following items:

- Dry yeast.
- Sugar (sucrose).
- Thermometer to measure water temperature.
- 2 empty plastic bottles.
- 1 cap (must fit both bottles).
- Aquarium aerator pump.
- Epoxy or silicone sealant.
- Graduated cylinder (an empty plastic bottle can be substituted, if necessary).
- Plastic tub or bucket.
- Packing tape.

Fermentation technology Lab. 7

Solid State Fermentation

Production of Protease by *Aspergillus niger*

Introduction

Proteases are a group of enzymes that catalyzes the hydrolysis of the peptide bonds of target proteins. They are also called proteolytic enzymes or proteinases. Proteases differ in their ability to hydrolyze various peptide bonds. Each type of protease has a specific kind of peptide bonds that breaks. Examples of proteases include: fungal protease, pepsin, trypsin, chymotrypsin, papain, bromelain, and subtilisin

The common protease producing microbes are *Aspergillus sp., Aeronomas sp., Alcaligenes sp., Bacillus sp., Staphylococcus sp.* and *Pseudomonas sp.*

The present uses of the protease in industrial applications are as:

(a) biological detergents

(b) Baking—dough modification/gluten weakening and flavour improvement. (c) Beer brewing.

(d) Leather manufacture.

(e) Cheese manufacture—clotting of milk protein and.

(f) Meat manufacture.

(g) Flavour control and production in food products.

(h) Waste treatment, e.g. recovery of silver from spent photographic film.

Solid State Fermentation (SSF) is the cultivation of micro organisms under controlled conditions in the absence of free water. It has emerged as a potential technology for the production of microbial products such as industrial enzymes, nutrient enriched animal feeds, fuel, food, industrial chemicals and pharmaceutical products. SSF systems offer numerous advantages over submerged fermentation (SmF) system, including high volumetric productivity, relatively higher concentration of the products, requirement for simple fermentation equipments, easier to meet aeration requirements and easier downstream processing.

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Lab no.: 8 Solid state fermentation (SSF)

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi, to produce a wide variety of substances that are highly beneficial to individuals and industry like antibiotics, peptides and enzymes, in addition to the usual products of fermentation, such as carbon dioxide and alcohol.

There are two broad fermentation techniques have gained importance due to their economic and environmental advantages: Submerged Fermentation (SmF) and Solid State Fermentation (SSF).

Solid-State Fermentation (SSF)

This process involves the fermentation of solid substrate medium with microorganism in the absence of free flowing water.

SSF utilizes solid substrates, like wheat bran, rice and rice straw, hay, fruit and vegetable waste, paper pulp, bagasse and bran. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates.

SSF is suited for fermentation techniques involving fungi and a number of bacteria requiring less moisture content. However, it cannot be used in fermentation processes involving organisms that require high water activity (a_w), such as most of bacteria. Thus, it is crucial to provide optimized water content, and control the (a_w) of the fermenting substrate for; the availability of water in lower or higher concentrations affects microbial activity adversely.

In SSF, microbial growth and product formation occurs at or near the surface of the solid substrate particle having low moisture contents and the microbe is in contact with atmospheric oxygen unlike in Submerged Fermentation.

SSF are normally multistep processes involving the following steps:

1- Pre-treatment of substrate raw materials either by mechanical, chemical

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Lab no.: 9 (Fermenter) Bioreactor:

Bioreactor: device, usually a vessel, used to direct the activity of a biological catalyst to achieve a desired chemical transformation.

Fermenter: type of bioreactor in which the biocatalyst is a living cell**.** Its refer to any devise that provides and maintains an optimum environment for the operation of fermentation. The fermenter is used to grow bacteria or fungi whereas bioreactor is used for eukaryotic cells such as plant cells. They also differ in the types of agitator and mixers used in them .

Proper design of ferment is essential for:

1. Maintaining suitable environment required for controlled growth of the cells. 2. Maintaining septic condition and prevent contamination.
3. Regulation certain aspects like agitation, pH and aeration etc.
4. Easy isolation and purification for the desired product.
5. Easy cleaning of equipment.

Classification of fermenters:

A- Based on size:

1- Laboratory (1-50 L).
2- Pilot plant (50-1000 L).
3- Production (> 1000 L).

B- Based on the type of growth system:

1- Submerged fermentation.
2- Solid state fermentation.

Components of fermenter:

1- Culture vessel: commonly cylinder, range in size from one liter to many cubic meters and are often made of stainless steel or glass. The vessel is sterilized in an autoclave.

Lab: 10

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