MEDICAL, FOOD& INDUSTRIAL MICROBIOLOGY (DMBL22)

(MSC - MICROBIOLOGY)

ACHARYA NAGARJUNA UNIVERSITY

CENTRE FOR DISTANCE EDUCATION

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M.Sc. MICROBIOLOGY (Final)

Practical-IV

MICROBIAL GENETICS, MOLECULAR BIOLOGY AND FOOD AND INDUSTRIAL MICROBIOLOGY

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Experiment No.16

SCREENING OF MICROORGANISMS FOR THE PRODUCTIONOF ORGANIC ACIDS

Aim

To screen different microorganisms for the production of Organic acids

Requirements

Calcium carbonate (0.5%) containing Czapek-Dox agar medium and nutrient agar medium, inoculation needle, spirit lamp and petridishes.

Procedure:

- Add 0.5% calcium carbonate to the Czapek-Dox agar medium and nutrient agar medium.
- Sterilize the medium at 121^{o}C temperature and 15 lb pressure for 15 minutes.
- Prepare calcium carbonate containing agar plates separately for the above media.
- Inoculate the fungal cultures in Czapek-Dox agar medium and incubate for 7 days at 28^{o}C.
- Streak the bacterial cultures onto the nutrient agar medium and incubate for 24 hours at 37^{o}C.
- Maintain control plates without inoculating any microorganism and incubate.
- At the end of incubation period, observe the clear zones around the colonies.

Observation

Clear zones around the colonies indicate the solubility of calcium carbonate due to production or organic acids by microorganisms.

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Experiment No.17

SCREENING OF MICROORGANISMS FOR THE PRODUCTION OF AMYLASES

Aim: To screen different microorganisms for the production of amylases.

Requirements: Starch (1%) containing Asthana and Hawker's medium A, nutrient agar medium, inoculation needle, spirit lamp, Gram's iodine solution and dropper.

Asthana and Hawken's medium A (pH 5-6)

Starch	..	10.0 g
Sodium nitrate	..	3.5 g
Potassium dihydrogen phosphate		1.75 g
Magnesium sulfate	..	1.50 g
Agar	..	20.0 g
Distilled water	..	1 litre

Procedure

- Add starch to small amount of water and boil for 10 minutes with constant stirring until it becomes solution and then add to the Asthana and Hawker's medium A and nutrient agar medium.
- Sterilize the medium at 15 lb pressure for 15 minutes.
- Prepare starch agar plates separately for the above media.
- Inoculate the fungal culture in Asthana and Hawker's medium A and incubate for 7 days at 27^oC to 29^oC.
- Streak the bacterial culture onto the nutrient agar medium and incubate for 24 hours at 37^oC.
- Maintain the control plates without inoculating bacteria and fungi and incubate at respective temperatures.
- At the end of incubation period, flood all the petridishes with Gram's iodine solution and keep for 5 minutes.
- Decant excess amount of Gram's iodine solution. Observe the decolourised zones around the colonies.
- Measure the decolourised area around the colony of each organism and express the results in millimeters.

Observation

Colourless clear zones around the colonies indicate the hydrolysis of starch due to production of amylases. The medium of control plates, on iodine treatment, turn to dark blue colour without any discoloration.

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Experiment No.18

SCREENING OF MICROORGANISMS FOR THE PRODUCTION OF ANTIBIOTICS

Aim: To screen antibiotic producing microorganisms by crowded plate method.

Requirement: Soil sample, trypticase soy agar medium, petriplates, pipettes, oven and bunsen burner.

Trypticase soy agar (pH 7.3)

Trypticase	15.0 g
Phytane	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	1 litre

Procedure

- Take 10 g of soil and place in 100 ml sterilized water taken in 250 ml Erlenmeyer flask.
- Shake the flask vigorously on a rotatory shaker for 30 minutes, so as to disperse soil particles. Allow the flask to stand for 30 minutes so that heavy soil particles settle down.
- Prepare serial dilutions from the stock solution by pipetting 1 ml and adding to 9 ml sterile water in a test tube aseptically and shake. Repeat the process to get dilutions of soil suspension (10^{-2}, 10^{-3}, 10^{-4}).
- Pipette out 1 ml from each dilution into sterilized petridishes and pour molten trypticase soy agar by rotating the plate gently to ensure uniform mixing of soil suspension with the medium. Allow the medium to solidify.
- Incubate the plates at room temperature for 2-4 days.

Observations and Results

Observe and identify the colonies with a zone of inhibition around them, which gives a positive indication for the production of antibiotic by those cultures.

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Experiment No.19

ESTIMATION OF CITRIC ACID PRODUCED BY MICROORGANISMS

Aim: To estimate the amount of citric acid in a culture broth of *Aspergillus niger*.

Requirements: *Aspergillus niger*, Acetic anhydride, Pyridine, colorimeter, Czapek-Dox broth, pipettes.

Procedure:

- Culture *A. niger* on Czapek-Dox broth for one week at room temperature and collect the culture broth.
- Take 2 ml of culture broth; add 2 ml of pyridine and 5 ml of acetic anhydride slowly through the sides of test tube taking care to avoid bumping.
- Incubate the reaction mixture for 30 min.
- Read the intensity of yellow colour thus developed in a colorimeter at 420 nm.
- Two ml of water in place of culture broth serves as blank.
- Read the amount of citric acid present in the broth with the help of standard curve.

Preparation of Standard curve:

- Prepare citric acid of different concentrations. Add 2 ml of pyridine slowly through sides of the tube. Take care to avoid bumping. Incubate all the tubes for 30 minutes to develop yellow colour. Read the intensity of colour at 420 nm in a colorimeter. Plot the graph taking optical density against concentration of citric acid.

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Experiment No. 20

ESTIMATION OF AMYLASE PRODUCED BY MICROORGANISMS

Aim: To estimate the amount of amylase produced by *Aspergillus niger*.

Requirements:

- 0.1 M sodium acetate buffer, pH 4.7.
- **1% starch solution:** Prepare a fresh solution by dissolving 1 g starch in 100 ml acetate buffer. Slightly warm, if necessary.
- Dinitrosalicyclic acid reagent (Dissolve simultaneously 1 g of dinitrosalicylic acid, 200 mg of Crystalline phenol and 50 mg of sodium sulphite in 100 ml of 1% NaOH solution by stirring).
- 40% Potassium sodium tartrate.
- Maltose solution (Dissolve 50 mg maltose in 50 ml distilled water in a volumetric flask).
- Czapek-Dox broth containing 1% starch.
- *Aspergillus niger* culture.

Procedure

- Inoculate 100 ml of sterilized Czapek-Dox broth taken in 500 ml conical flasks with 2 ml of spore suspension (~ 4000 spores per ml) of *Aspergillus niger*.
- Filter the mycelium using Whatman No.1 filter paper and centrifuge the filtrate at 2000 g for 30 min to remove the spores. Use the supernatant as enzyme source.
- Pipette out 1 ml of starch solution and 1 ml of properly diluted enzyme in a test tube.
- Incubate at 27^{o}C for 15 min.
- Stop the reaction by the addition of 2 ml of dinitrosalicyclic acid reagent.
- Heat the solution in a boiling water bath for 5 min.
- While the tubes are warm, add 1 ml of potassium sodium tartrate solution.
- Then cool in running tap water.
- Make up the volume to 10 ml by addition of 6 ml water.
- Read the absorbance at 560 nm.
- Terminate the reaction at zero time in the control tubes.
- Prepare a standard graph with 0-100 μg maltose.

Calculation:

A unit of α or β-amylase is expressed as mg of maltose produced during 5 min incubation with 1% starch.

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Experiment No.21

ESTIMATION OF STREPTOMYCIN PRODUCTION

Aim: To estimate the amount of streptomycin produced in culture broth of *Streptomyces* species.

Requirements: Stock solution of 10% ferric chloride in 1N HCl, 1N NaOH, 1.3 N HCl and culture broth of *Streptomyces* species.

Procedure:

Preparation of standard graph

- Dissolve 100 mg of streptomycin in 100 ml distilled water. Make serial dilutions from this stock solution as desired.
- Take one ml of streptomycin solution from each dilution and add 1 ml of 1N NaOH. Heat the tubes in water bath for 10 minutes and cool in ice water.
- Add 1 ml of 1.3 N HCl to the reaction mixture, then add 2 ml of 10% ferric chloride to each tube. One ml of distilled water in place of streptomycin solution and all other reagents serve as blank.
- Read the intensity of colour thus developed at 550 nm. Plot the graph for concentration of streptomycin against optical density.

Estimation of streptomycin:

- Take one ml of suitably diluted culture broth and add 1 ml of NaOH, heat for 10 minutes and cool immediately in ice water.
- Add one ml of 1.3 N HCl and 2 ml of ferric chloride.
- Read the intensity of colour thus developed at 550 nm.
- Calculate the amount of streptomycin from a standard curve and express in mg/ml.

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Experiment No.22

PENICILLIN PRODUCTION

Aim: To assay the production of Penicillin by *Penicillium chrysogenum*.

Requirements: Penicillin producing strain of *Penicillium chrysogenum*, Czapek-Dox broth medium, Inoculating needle, Bunsen burner.

Procedure:

Aseptically inoculate the Czapek-Dox broth medium with culture of *P. chrysogenum.*

Incubate the culture at 25-28^{o}C for 7-14 days.

Observations and Results:

The development of a golden yellow colour in the medium or the droplets on the mycelium will indicate the production of active penicillin by the mold.

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Experiment No. 23

F E R M E N T O R

Fermentors are used to culture the microorganisms. The main function of a fermentor is to provide a controlled environment for the growth of microorganisms to obtain a desired product which may be cell biomass, a metabolite or bioconversion product. The most commonly used fermentors are Stirred Tank Reactors (STRs). They are produced in a range of sizes from one dm^3 to thousands of dm^3. STRs have been adopted for a wide range of fermentation processes (Fig. 1).

Glass and/or stainless steel may be used for constructing the body of the fermentor. Pilot-scale and industrial scale vessels are normally constructed with stainless steel or at least have stainless steel cladding to limit corrosion. The fermentor should be provided with systems for temperature control, pH control and for sampling facility.

The fermentor should provide adequate aeration and agitation. The main purpose of aeration is to provide microorganisms in submerged culture with sufficient oxygen for metabolic requirements. Agitation is required to maintain a uniform environment through out the vessel contents. Disc turbines are the commonly used agitators which consist of a disc with a series of rectangular vanes set in a vertical plane around the circumference. Baffles are metal strips attached radially to the wall of fermentor. Four baffles are normally arranged to improve aeration efficiency and to prevent vortex.

Sparger is used to introduce air into the medium in a fermentor. Valves attached to fermentors are used to control the flow of liquids and gases in several ways. The addition of inoculum, nutrients and other supplements must be carried out under aseptic conditions. Aseptic sampling is performed using a steam-locked port.

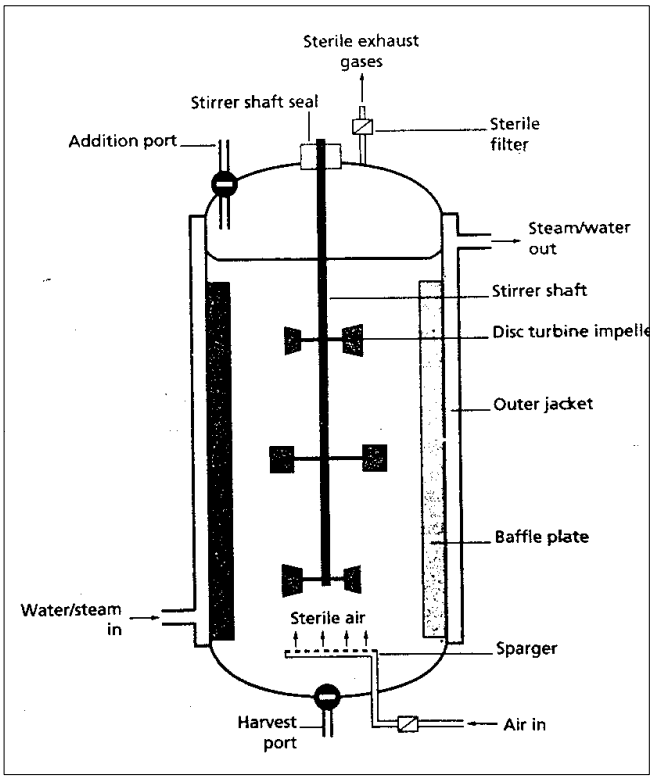

Fig. 1. Stirred tank Reactor

Air-lift fermentor

An air-lift fermentor contains a gas tight baffled riser tube (liquid ascending) connected to a down comer tube (liquid descending). Air-lift fermentors with an external riser and internal riser are shown in Figures 2 and 3 respectively.

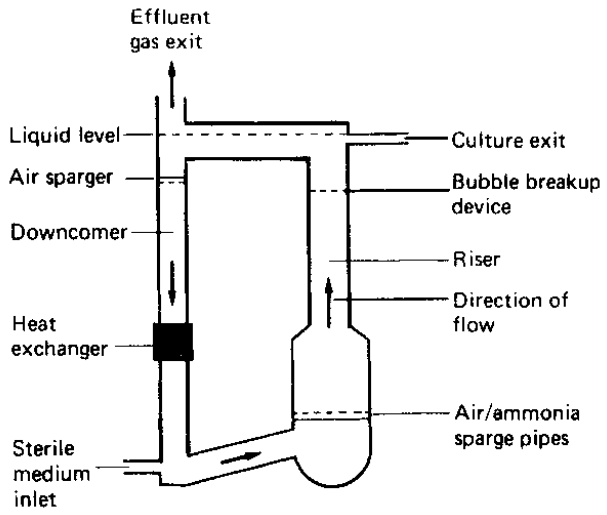

Fig. 2 Air-lift fermentor with outer loop

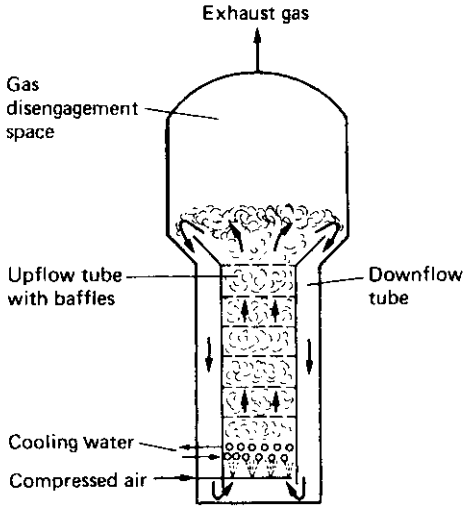

Fig. 3 Air-lift fermentor with inner loop

The advantage of the air-lift systems is that it has no moving parts making operation and maintenance easier. In this fermentor air is introduced into the base of the riser by a sparger. The driving force for circulation of medium in the vessel is produced by the difference in density between the liquid column in the riser (excess air bubbles in the medium) and the liquid column in the downcomer (depleted in air bubbles after release at the top of the loop). All performance characteristics of air-lift fermentors are linked to the gas injection rate and the resulting rate of liquid circulation. Air-lift bioreactors are highly energy-efficient relative to stirred fermentors, yet the productivites of both types are comparable.

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