M.Sc. Microbiology **2018-2019**

18MBP312 **APPLICATION ORIENTED PRACTICAL – VI** **Semester – III**
4H – 2C

Instruction Hours / Week : L: 0 T: 0 P: 4 **Marks:** Internal: **40** External: **60**
Total:**100**

End Semester Exam: 9 Hours

COURSE OBJECTIVES

This provides information on fermented food product production in food industries. To know the possible contamination of food products which may include bacteria and fungi.

COURSE OUTCOME (CO'S)

This practical adds a good understanding of industrial microbiology and become qualified as microbiologist in food and beverage industries.

1. Production of enzymes – solid state & submerged fermentation – Amylase and protease.
2. Production of sauerkraut ,yoghurt, wine
3. Enumeration of Microorganisms from Food samples
4. Detection and enumeration of Microorganisms present in lab surfaces.
5. Analysis of Milk quality by MBRT and resazurin
6. Detection of coliforms from water - MPN test
7. Isolation of plant pathogens – Bacteria and fungi
8. Citric Acid production
9. Mushroom Cultivation
10. Immobilization technique (Sodium alginate method).

SUGGESTED READINGS
REFERENCES

1. Adams, M.R., and Moss, M.O., (2000). *Food Microbiology*. Royal Society of Chemistry. Cambridge, U.K.
2. Ahmed, E.Y., and Carlstrom, C., (2003). *Food Microbiology: A Laboratory Manual*, John Wiley and Sons, Inc. New Jeresy.
3. Arora, B., and Arora, D.R., (2007). *Practical Microbiology*. (1st ed.). CBS Publishers and Distributors, Bangalore.
4. Cappucino, G.J., and Sherman, N., (2001). *Microbiology A Laboratory Manual*. (6th ed.). Benjamin Cummings, New York.
5. Demain, A.L., and Davies, J.E., (1999). *Manual of Industrial Microbiology and Biotechnology* (2nd ed.). ASM Press, Washington.
6. Garg, N., Garg, K.L., and Mukerji, K.G., (2010). *Laboratory Manual of Food Microbiology*. I.K. International Publishing House, New Delhi.
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PRODUCTION OF ENZYMES – SOLID STATE & SUBMERGED FERMENTATION – AMYLASE AND PROTEASE.

AIM:

To produce the enzyme amylase and protease by fermentation technique using microorganisms.

INTRODUCTION:

Amylase is an enzyme that breaks down starch or glycogen. Amylase is produced by variety of living organism ranging from bacteria to plants and humans. Bacteria and fungi secrete amylase to outside of their cells to assay out extracellular digestion when they have broken down the insoluble starch into soluble end product such as glucose or maltose are absorbed into their cells.

SOLID STATE FERMENTATION:

Solid State fermentation is manufacturing process used in the production of fuel food, pharmaceutical and industrial products. It is used as an alternative submerged fermentation. It is known in Japan as Koji fermentation and has existed for many years. It is the use of microorganism in a controlled environment to produce enzymes, fuel and nutrients. Solid State fermentation occurs in absence of free water. There are a number of advantages in the use of solid State fermentation over submerged fermentation. It is much more simple process which requires a lot less energy. It produce a much higher volumtric productivity and it is similar to the natural environment of certain fungi. The solid state process involves a solid matrix like rice bran and placing than submerged fermentation. It is more easy and placing it on a medium to alongside microbes create a substrate. This is then stored at a specific temperature between 5 and 95°c for 1-5 days. It is also subject to agitation using constant or intermittent rotation.

SUBMERGED FERMENTATION:

Submerged fermentation is a process involving the development of microorganisms in a liquid broth. Thisliquid broth contains nutrients and it's results in the production of industrial enzymes, antibiotics or other products. The process involves taking a specific microorganism and placing it in a small closed flask containing the rich nutrient broth. A high volume of oxygen is also required for the process. The production of enzyme then occurs when the microorganism interact with the nutrients on both resulting in them being break down.

MATERIALS REQUIRED:

Autoclave, microwaveoven, nutrient agar,PDA, soluble starch, Shaker, spectrophotometer, water-bath, disposable spoons, micropipette , sterile water,petridishes, inoculation loop, Needle or bore,Bunsen burner, glass spreader,95% ethanol.

AMYLASE PRODUCTION MEDIUM:

Bacteriological peptone eg:mgso4(0.5g),kcl(0.5g),starch (1g) sterlized by autoclaving at 121°c for 15 mins.

PROCEDURE:

ISOLATION of AMYLASE PRODUCERS:

Collect 100g of top soil and transfer into a ziploe bag.Suspended about 10g of soil in 100 ml steriledistilled water properly (10-1).

Pipette 10 ml of the above and transfer to another 90ml of water (10_2). Dilute further in two more 90 ml sterile water blanks (10_3 and 10_4).

Separate 0.1ml of diluted samples (10_1 and 10_2) on nutrient agar plate (4 plates) containing 1%w/v of soluble starch and incubated at 30°c for 24 hrs.

Starch hydrolysis clonies will have an area of clearance around them.

Transfer distinguishable amylase producing bacteria by streaking on a fresh plate of N.A containing 1%starch.

AMYLASE PRODUCTION:

1. SEED CULTURE:

Prepare amylase production medium and sterile properly.

Inoculate loopfull amylase producing the bacillus sps.incubate for 24 hrs at at 37°c.

2.FERMENTATION:

Prepare 250 ml of AMYLASE PRODUCTION MEDIUM in 500ml conical flask.Sterile at 121°c for 15 mins.

Inoculate 25 ml of seed culture.incubate at 37°c for 24 hrs under shaking condition.withdraw culture and subject to extraction of enzyme.

3.EXTRACTION OF ENZYME FROM BACTERIA:

Pour the bacterial culture into centrifuge tubes and spin for 20 mins at 5000 rpm.

Decant the supernatant and collect the crude enzyme extract in a sterilized beaker.

4.ENZYME ACTIVITY:

1ml of strach solution and 1 ml of properly diluted enzyme is pippete in a test tube .It is incubated at 27°c for 15 mins.

The reaction is stopped by the addition of 2 ml of dinitro salicylic acid reagent.

The solution is then heated in a boling water for 5 mins...

While the tubes are warm,add 1ml of potassium sodium tartarate solution.

Then cool it in running tap water.The volume is made to 10 ml by the addition of 6ml distilled water.The absorbence is read at 560ml.

RESULT:

When the plates were flooded with lugals iodine solution clear zone was observed around the colony.This indicated the population of the AMYLASE enzyme.

DISCUSSION:

Amylase is a hydrolytic enzyme which breaks down starch to yield maltose as end product.

$(C6H12O6)n + H2$----------> $n(C6H2O)$ maltose

In any enzyme assay the rate of reduction can be known by reusing the amount of substrate that utilized by the amount of product that is maltose. Both the components are coloured test estimated directly. The reducing sugar produced by the action of amylase, react with dinitosalicylic acid, the intensity of color depends on the concentration of maltose.

PRODUCTION OF PROTEASE

Aim

To produce protease from microorganism

Materials and methods

Rhizopus microsporus var oligospous was procured from the institute of Microbial Technology, Chandigarh and was maintained at 4°C on potato dextrose agar (PDA). Spore suspension was made from five days old cultures that had been grown on PDA slants at 30°C. It was prepared by suspending the spores from one tube in 10 ml of sterilized distilled water containing 0.1ml Tween–80. The spore count was adjusted to105 spores ml-1. One ml of inoculum was used per flask to carry out submerged fermentation.

Shake flask cultivation media The enzymes were produced using basal medium. The basal medium contained the following ingredients (g l -1): (NH4) SO4 - 1.4, CO(NH2)2 - 0.3, KH2PO4 - 4.0, K2HPO4 – 0.84, CaCl2 .2H2O – 0.3, MgSO4 .7H2O – 0.3, FeSO4 .7H2O – 0.005, MnSO4 .H2O – 0.00156, ZnCl2 – 0.00167, Peptone 0.25, Yeast extract – 0.10, Rice Bran – 4%. All the components of the basal medium except rice bran were dissolved in distilled water. Flasks having 250 ml capacity were taken and 50 ml of this solution was poured in each flask. To each flask 2.0 g of rice bran was added and autoclaved at 1.1kg/cm2 for 20 minutes. Flasks were cooled and inoculated with 1ml of the spore suspension. The inoculated flasks were incubated at 30°C on a rotary shaker cum BOD incubator revolving at 150 rpm. After 96 hours of growth, the contents of each flask were centrifuged at 4°C and this supernatant was used as the source of crude enzyme.

Partial purification of fungal protease

Protease was isolated and partially purified with isopropanol. The mold filtrate (20 ml) was chilled at 4°C in a refrigerator and 25 ml of chilled isopropanol was added to it. The precipitates were separated by centrifugation. The precipitates were dissolved in 5 ml of 0.05 M citrate phosphate buffer having pH 5.2 to obtain partially purified enzymes.

Protease activity

The test tubes containing 1ml casein solution and 1ml enzyme extract were incubated at 60°C for 10 minutes. After 10 minutes 3 ml TCA was added to each test tube to stop the reaction. The precipitates formed were centrifuged at 5000 rpm. The protein concentration in the supernatant was determined by Lowry's method. Optical density was recorded at 525 nm. Protease activity was expressed as Tyrosine equivalents using the standard curve prepared for measurement of proteins under same set of conditions as described above using standard solution of Tyrosine. Microorganisms and production of protease Protease producing B. licheniformis was grown on nutrient agar at 37°C for 24 h for inoculum preparation. A loopful of the growth was transferred to to Laura broth (LB) liquid medium (1% yeast extract, 0.5% peptone, 0.5% NaCI, (w/v), pH 7.0). One hundred milliliters of LB was inoculated with 1mL of the inoculum (3x106 cells=mL) and was incubated at 37°C for 24 h. After incubation, the crude enzyme was obtained by centrifugation of the culture broth at 10,000xg for 10 min at 4°C. The cell-free supernatant containing the enzyme was assayed for protease activity. Enzyme assay Protease activity was determined using sulphanilamide azocasein. The reaction mixture containing 250 µl 1% (w/v) substrate in 0.1M Tris/HCI buffer (pH 9.0) and 150 µl of enzyme solution was incubated for 30 min at 37°C. After incubation, the enzyme was inactivated by addition of 1.2 ml trichloroacetic

acid solution (10%, v/v) and then the solution was neutralized using 800 µl of 1.8 N NaOH solution. The absorbance was read at 420 nm. One unit of proteolytic enzyme activity was defined as the amount of azocasein that hydrolyzed during 30 min incubation at 37°C for millilitre of solution of extract. All experiments were conducted in triplicate and the mean at three with standard deviation (SD) was represented. Assay of protein concentration. The protein concentration was determined by the Lowry method by using bovine serum albumin as standard. The effect of incubation period was determined by incubating production medium for different incubation periods (12, 24, 48, 72, 96, and 120 h) at 37°C taking other conditions into consideration.

PRODUCTION OF SAUERKRAUT ,YOGHURT, WINE

Production of Sauerkraut

Aim:

To become acquinated with tha microbiological production of Sauerkraut.

Principle:

Sauerkraut is a classic example of a food of plant Origin produced by microbiological fermentation. Its preparation requires the fermentation activities of a mixed microbial flora. Including Leucanostoc, Mesenfureides,Lactobacillus,Plantarum c.blevis,enterococcus fecalis.

In the production of Sauerkraut,shreded cabbage is treated with Nacl which creates an osmotic environment in which plasmalysis occurres extracting the juice from the cabbage tissue. The lactic acid is responsible for the characters Sauerkraut flavour and also act as a preservatives by inhibiting the growth of microorganisms that cause food spoilage.

Production of the lactic acid is iniciated by L.mensenteroids,which are cocci and sustained by L.plantarum which are bacilli. When the acid concentration reaches a level of 0.7% to 0.1% . The fermentation activities of L.mesenteroides cause and the final stage of the process are caused out by L.plantarum and L.blevis and E.fecalis. This finished product contains a total acidity of 1.5% to 2% of which lactic acid represents 1% to 1.5%.

Characteristics:

1) Odour: Acid earthy , spicy or patri

2) Colour: Brown pink, straw yellow, pale yellow or colourless

3) Texture: a) Soft - Fermentation irritated by L.plantarum rather than L.mesenteroids.
b) Stimy - rapid growth of L.cuecumecis at elevated temperature.
c) rotted - spoilage by bacteria , yeast or mold.

4) pH: The ph of the finished product should be in the range of 3.1 to 3.7.

Place 10ml of the fermentation juice and 10ml distilled water into an Erlenmeyer flask. Boil to clear the co2

Cool and added 5 drops of 1% phenol to the distilled juice.

Titrate to the first presistant sample which pink colour with 0.1N NaOH

Calculate % lactic acid as follows:

%lactic acid = ml of alkaline × normality of alkaline / weight of the sample in g ×g

1ml = 1g

Microscopic appearance of the microbial flora.

Materials required:

1) Reagents: 1% phenolphthalein, 0.1N NaOH, Methylene blue and uniodized table salt.

2) Equipment: Two wide mouthed jars with covers. Two wooden boards to fil into jars two heavy weights chees cloth, bunsen burner , inoculating loop, glass slides, coverslip, 10ml disposable pipettes, mechanical pipetting device knife and Erlenmeyer flask.

Procedure:

1) Remove the outer leaves and all bluished tissues from each of the cabbage heads.

2) Thoroughly was the head in the tap water

3) Weight the sheeded cabbage on a balance and separate into equal portions

4) Weight out the table salt in amounts equal to 3% of the each of the potions sheeded cabbage.

5) Place a wooden board over each of the mixture and press gently to squeeze out a layer of juice from cabbage.

6) Place a weight on each of the boarders and cover the jars with cheese cloth.

7) Incubate the jars for 14 days at 30℃.

Result:

Prepared Sauerkraut is subjected to microbial examination.

Production of yogurt

Aim

To prepare yogurt from milk using *Lactobacillus* and *Streptococcus*

Introduction

Yogurt is produced by the fermentation of warm milk by *Lactobacillus bulgaris* and *Streptococcus thermophillus*. These two bacteria are able to grow at 40-45°C during which they produce lactic acid and various other by products that give its unique flavor.

Materials required

Cow milk, concentrated skim milk, non fat dry milk, whey lactose,
sweatners – glucose and sucrose, high intensity sweetners eg) aspartains.
Stabilizers – gelatin, carbonyl methyl cellulose, lowest sugar, alginates, whey protein concentrate, starts culture, *Strptococcus salvarius* subsp thermophilus and *Lactobacillus delburki*

Procedure

100ml of the whole milk was taken in 500ml sterile beaker and boiled.
4gm of powdered milk was added and stirred constantly using sterile glass rod.
Required quantity of sweetener and stabilizer and flavoring agent was added.
Milk sample was cooked at 45°C and inoculated with 1 teaspoon of commercial yogurt or 10ml of culture.
The inoculated sample was wrapped and incubated at 45°C for 24 hrs.
RESULT:

The product was observed for texture and taste.

PRODUCTION OF WINE

AIM

To become acquainted with wine production by the fermentative activities of yeast cell .

PRINCIPLE

Wine is a product of natural fermentation of the juices of grapes and other fruits such as peaches, pears, plums and apples by the action of yeast cell. The biochemical conversion of juice to wine occurs when the yeast cell enzymatically degrade the fruit, sugars, fructose and glucose first to acetaldehyde and then to alcohol.

Grapes containing 20%-30% sugar concentration Will yield wines with an alcohol content of approximately 10%-15%. For red wine the crushed grapes must be fermented with their skin to allow extraction of this colour in to the juice. White wine is produced from the juice of white grapes and skin removed red grapes.

The commercial production of wine is along and extracting process. First the grapes are crushed or pressed to express the juice which is called must potassium metasulfide is added to the must be retard the growth of acetic acid bacteria, molds and yeast that are endogenous to grapes in the

wine yard. A wine producing strain of yeast Saccharomyces cervisiae var ellipsoideces is used to inoculate the must which is then incubated 3-5 days under aerobic condition at 21*c-32*c. This is followed by an anaerobic incubation period. If any turbidity there by producing volatile esters that are responsible for the characteristic flavours.Total acidity (expressed as 1% tartaric acid). To a 10ml aliquote of the fermenting wine, add 10ml of distilled water and 5drops of 1% phenolphthalein solution. Mix and titrate to the first persistant pink colour with 0.1N NaoH.

TOTAL ACIDITY

1. % tartaric acid=ml alkaline * normality. *7.5./weight of sample in g.
2. Volatile acidity (expressed as 1%acetic acid) :following titration, calculate volatile acidity using the formula.

%acetic acid =ml alkaline * normality * 6/weight of sample in g

3. Alcohol (expressed as volume %)
4. Aroma: fruity, yeast like, sweet
5. Clarity: clear turbid.

MATERIALS REQUIRED

Culture: 50ml of grape juice broth culture of Saccharomyces cerevisiae var ellipsoidetes incubated for 48hrs at 25*c

Media: 500ml of pasteurised commercial white grape juice.

Reagents: 1% phenolphthalein solution. 0.1N NaoH, sucrose

Equipment : 1c erlenmeyer flask, one holded rubber stopper containing a 2 inch glass table plugged with cotton, pan balance, spatula, glass Inc paper and burette or pipette for titration.

PROCEDURE

Pour 500ml of the grape juice in to the ic erlenmeyer flask. Add 200g of sucrose and 50ml of saccharomyces cervisiae grape juice broth culture.

After 2 days and 4 days of incubation, add 20 g of sucrose to the fermenting wine

Incubate the fermenting wine for 21 days at 25*c.

RESULT

Taste and nature of wine is good.

ENUMERATION OF MICROORGANISMS FROM FOOD SAMPLES
Isolation of food borne bacteria from food products

Isolation of spoilage microorganisms from bread

Aim:

To isolate and distinguish the spoilage of microorganisms from bread.

Principle:

Foods are more susceptible to microbial decomposition because they are rich in nutrients that support the growth of microorganisms. Contaminated foods are often inedible and may also be the source of human diseases if contaminating organism are pathogens or toxin producers. Some of the important food poison produced by bacteria is botulism (caused by Clostridium botulinum), Staphylococcal poisoning (Staphylococcus aureus) and enteric (E. coli) and (Salmonella typhimurium). This food poisoning is serious and sometimes fatal. Molds are most common cause for spoilage of bread. The temperature attained in the baking procedure is usually high enough to kill the air during cooling from handling or from wrapper and usually initiate growth in the crease of the loaf and between the slice of the bread. Chief molds involved in the spoilage of bread are the bread molds, Rhizopus stolonifer, other molds are Pencillium expansum or Pencillium stolonifer, Aspergillus niger etc. Ropiness of bread is common in homr baked breads, especially during hot weather. It may be caused by bacterial species namely Bacillus subtilus or Bacillus licheniforms.

Materials required:

Sample: Bread

Medium: Nutrient agar medium for bacterial isolation and Potato dextrose agar for fungal isolation, antibiotic (chloramphenicol).

Sterile petriplates, inoculation loop, sterile pipettes, mortar and pestles.

Procedure:

- About 1 gm of the spoiled bread to be tested was taken and placed in a clean mortar.
- The sample was then ground to a fine pulp with the help of a pestle.
- The sample was added into 99 ml of the sterile distilled water and considered as stock solution. Then 1 ml of the stock solution is added to 9 ml of sterile distilled water to obtain 10^{-1} dilution, the sample was serially diluted up to 10^{-7} dilution.
- Then spread plate technique was performed using nutrient agar plates for the bacterial isolation and potato dextrose agar for fungal isolation. 01 ml of the sample was used for spread plate technique.

DETECTION AND ENUMERATION OF MICROORGANISMS PRESENT IN LAB SURFACES

Aim:

To isolate the pathogenic microorganism from lab surface.

Introduction:

The laboratory environment harbours a heavy load of microbial flora.These surface are subjected to contamination by microorganism.some of which are able to form biofilms contamination of surface depends on their characteristics such as smooth, rough,porous or irregular and their state for example before or after the cleaning process,a new or old dry or wet.The lab is probably the most crucial area that harbours and transmit infection.Lab surface can contribute to the speed of bacteria such as E.coli, Klebsiella species,S.aures,Salmonella species and Camphylobacter species.

MATERIALS REQUIRED:

Nutrient agar,potato dextrose agar,petriplate,sterile cotton swabs etc.,

PROCEDURE:

1. Swab method is commonly used in lab surface to detect the pathogenic bacteria.
2. Swab is done by using a sterile cotton swab with an applicator stick for the detection of microorganism.
3. The cotton swab is applied on lab surface to recover the bacterial and fungal spores by plating method.
4. The plating was done by rubbing the swab on agar plate of nutrient medium for bacteria and PDA for fungi and incubate at 37 degree Celsius for 24 hours for bacteria and for 5days for fungi at room temperature.
5. Perform serial dilution technique and pour plate techniques for bacterial isolated spread plate was performed for fungal at room temperature.
6. The bacterial and fungal isolates were counted and the results were tabulated.

RESULTS:

The number of bacteria counted on nutrient agar plate is ---------Fungal colonies appeared on the potato dextrose agar plate is _____.

ANALYSIS OF MILK QUALITY BY MBRT AND RESAZURIN

Aim

To determine the quality of the given milk sample based on the difference in the microbial load milk sample provided.

Principle

Methylene blue and Resazurin reduction test depends on the fact that the color imparted to the milk by adding a dye will disappear more or less quickly that depends on the quality of the milk sample to be examined. Methylene blue and Resazurin is a redox indicator that loses its color under the absence of oxygen and is thought to be reduced. The reduction of oxygen in the milk is due to the production of reducing substance in the milk due to the production of reducing substance in the milk due to the enhanced rate of bacterial metabolism. The dye reduction time refers to the microbial load in the milk and the total metabolism reduction of the microorganism.

Materials and methods

Milk sample to be analyzed, screw cap tubes, test tube racks, pipettes (10ml and 1ml) and water bath (37 °C).

Preparation of methylene blue and Resazurin

The solution is prepared by boiling water in a brown stoppered flask the adding 1 methylene blue tablet to the flask of hot water and dissolve completely under hot condition. The solution may be stored in the stoppered brown flask to protect from sunlight.

Procedure

Transfer 10ml of each milk sample to approximately labeled test tube.

Add 1ml of redox indicator dye to each test tube containing the milk samples.

Tighten the test tube mouth with stopper gently invert at about 4 or 5 times to ensure proper mixing of the dye.

Heat the tubes in the water bath at 37 °C. Note the incubation time

DETECTION OF COLIFORMS FROM WATER - MPN TEST

Aim

To determine the quality of the given Water sample to check the potability.

MPN is most commonly applied for quality testing of water i.e *to ensure whether the water is safe or not in terms of bacteria present in it*. A group of bacteria commonly referred as fecal coliforms act as an indicator for fecal contamination of water. The presence of very few fecal coliform bacteria would indicate that a water probably contains no disease-causing organisms, while the presence of large numbers of fecal coliform bacteria would indicate a very high probability that the water could contain disease-producing organisms making the water unsafe for consumption.

Principle

Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas.The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction *(i.e both color change and gas production)* and comparing the pattern of positive results *(the number of tubes showing growth at each dilution)* with standard statistical tables.

MPN test is performed in 3 steps

1. Presumptive test
2. Confirmatory test
3. Completed test

1. Presumptive test:

The presumptive test, is a screening test to sample water for the presence of coliform organisms.

If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe.

If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction.

The method of presumptive test varies for treated and untreated water.

Requirements :

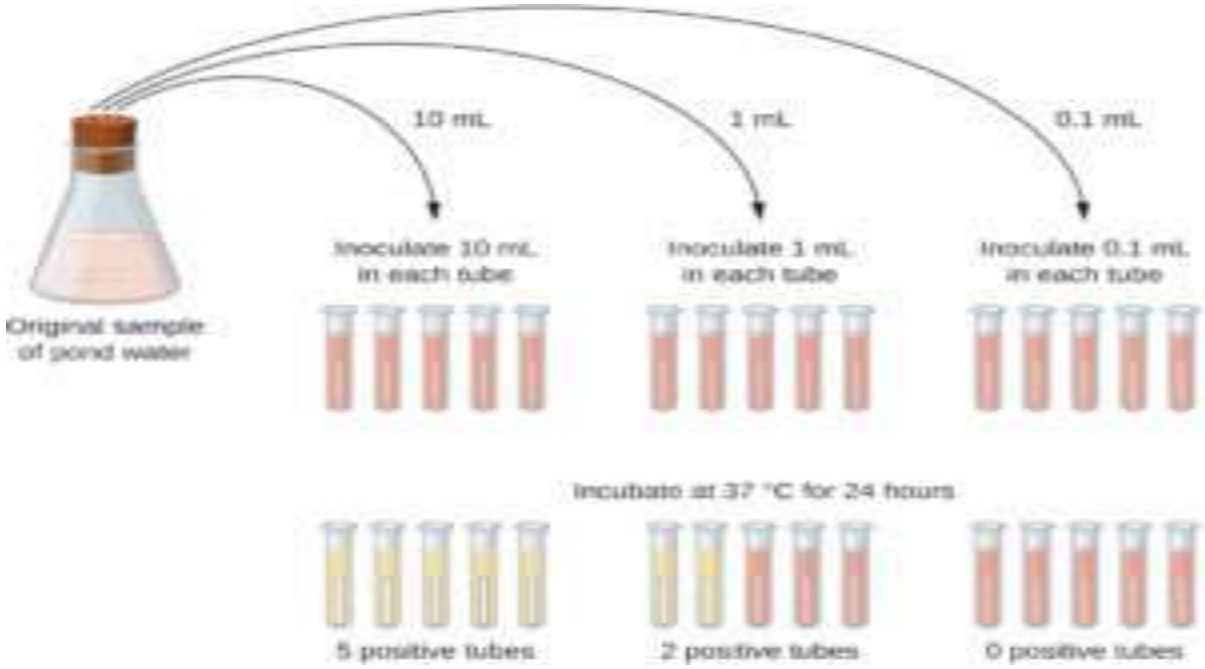
- Medium: Lactose broth or Mac Conkey Broth or Lauryl tryptose (lactose) broth
- Glasswares: Test tubes of various capacities (20ml, 10ml, 5ml), Durham tube
- Others: Sterile pipettes

Preparation of the Medium

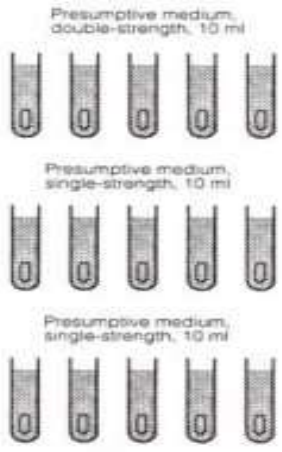
- Prepare medium (either mac conkey broth or Lactose broth)in single and double strength concentration.
- **For untreated or polluted water :**
  - Dispense the double strength medium in 10 tubes (10ml in each tube) and single strength medium in 5 tubes (10 ml in each tube)and add an durham tube in inverted position.
- **For treated water:**

- Dispense the double strength medium in 5 tubes (10ml in each tube) and 50 ml single strength medium in 1 bottle and add an durham tube in inverted position.
- Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.
- Sterilize by **autoclaving at 15 lbs pressure (121°C) for 15 minutes**.

Procedure of the test



A. For untreated water



MPN Water Testing

1. Take 5 tubes of double strength and 10 tubes of single strength for each water sample to be tested.
2. Using a sterile pipette add 10 ml of water to 5 tubes containing 10 ml double strength medium.
3. Similarly add 1 ml of water to 5 tubes containing 10 ml double strength strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml double strength medium.

4. Incubate all the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.
5. Compare the number of tubes giving positive reaction to a standard chart and record the number of bacteria present in it.
   For example: a water sample tested shows a result of 3–2–1 (3 × 10 ml positive, 2 × 1 ml positive, 1 × 0.1 ml positive) gives an MPN value of 17, i.e. the water sample contains an estimated 17 coliforms per 100 ml

MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when five 10-ml, five 1-ml and five 0.1 ml test portions are used)

No. of tubes giving a positive reaction :			MPN (per 100 ml)	95% confidence limits	
# of 10 ml	# of 1 ml	# of 0.1 ml		Lower	Upper
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46

ISOLATION OF PLANT PATHOGENS – BACTERIA AND FUNGI

Isolation of spoilage microorganisms from leaf and fruits

Aim:

To isolate and distinguish the spoilage microorganisms from leaf and fruits.

Principle:

The term 'pathogen' means the organism that incites diseases on living being. It may be fungus, bacteria, virus etc. The pathogen can easily be isolated in artificial culture media for identification and subsequent characterisation. Plants are more susceptible to microbial decomposition because they are rich in nutrients that support the growth of microorganisms.Infected plants are often inedible and may also be the source of human diseases if contaminating organism are pathogens or toxin producers.

Materials required:

1. Infected plant parts/plant
2. Petridishes
3. Forceps and knife
4. Mercuric chloride solution (1:1000)
5. Distilled water (steriled)
6. Slants with desirable culture media (Nutrient Agar and SDA)
7. Incubator
8. Bunsen burner
9. Slide and cover glass
10. Microscope
11. Absolute alcohol

Procedure:

(1) An infected plant or leaf of rice was brought to the laboratory from the field for isolation of pathogen.
(2) Initially the symptoms were examined under microscope or by hand lens. The infected lesionic part was removed by a knife and quickly transferred to the sterilising solution.
(3) Surface sterilisation of the infected plant was done by transferring the excised infected leaf segment into a petridish containing mercuric chloride solution (1: 1000) and kept for 2 – 3 min.
(4) Then the leaf segment was transferred to a series of petridishes containing sterile distilled water for washing of mercuric chloride.
(5) Finally the excised leaf segment was placed aseptically into the slant for culture of pathogen.
(6) The slant was then incubated at a required temperature for 3 – 5 days after proper leveling.
(7) Finally, the culture thus developed was examined microscopically.

Observation:

On microscopic examination of culture, conidia of Helminthosporium with conidiophores were noticed.

CITRIC ACID PRODUCTION

Aim:

To produce citric acid from micro organism using sugarcane molasses.

INTRODUCTION:

Citric acid is a weak organic acid with the fromula C6 H3 O7. It is neutral preservative used to add an acidic or sour taste to food and drinks to conjugate base of citric acid citrate is important as an intermediate in the citric acid cycle, which occurs in the metabolism of all aerobic organisms.It consists of three carboxy (R-COOH) groups. The most commercially used method for the production of citric acid is by Aspergillus Niger using cane molases as an example of fungal metabolism.

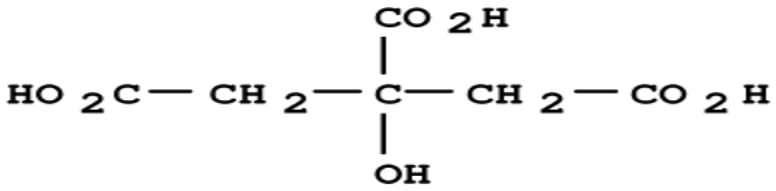


Figure 1. Chemical structure of citric acid.

Many microorganisms such as fungi and bacteria can produce citric acid but Aspergillus Niger remained the organisms of choice for the production organism of citric acid due to its genetic stability high yields and absence of undesirable reactions by recycling and resisting waste materials from Cane molasses.citric acid production can be easily achieved by using micro organism that has ability to produce citrc acid efficiency such as Aspergillus Niger.

PHYSICAL CHARACTERISTICS OF SUGARCANE MOLASSES:

Moisture content

Ash measurement

pH were analysed

MOISTURE CONTENT AND ASH MEAUREMENT:

The moisture content and ash measurement 2 molasses were performed by tacking log of molasses sample and dried in at 104 degree Celsius for 30 minutes.

Moisture content% =(A-x/A) *100

Ash unit = weight of molasses before burnity (A)- weight of molasses after burnity (X).

THE pH VALUE:

The pH value was measured before inoculation of molasses sample using pH metre device.

PRODUCTION OF CITRIC ACID FROM RAW SUGARCANE MOLASSES:

Aspergillus Niger transferred to the flash containing raw sugarcane molasses media with deferent concentration (20%,30%40%,50%,60%) .

An amount of 50 ml distilled water was added to be fungal pure culture to make a fungal suspension and then 10 ml from this suspension was transferred to be sugarcane molasses media.

DETECTION OF CITRIC ACID:

The detection of citric acid was done chemically by the addition of 3 drops of bromocresol green indicator to the 10 ml of distillation yield.

DETERMINATION OF CITRC ACID CONCENTRATION:

Citric acid was determined by titration using 0.1N Nacl and phenol, phenolphthalein as indicator and calculated as percentage according to the following formula,

Normality of citric acid =Normality of NaoH * NaoH / volume of citric acid.

Concentration of citric acid = Citric acid normality * equivalence * 100 / volume of distillation.

Equivalent = 96, Volume of distillation = 10.

RESULT:

The considered amount of citric acid was produced and thus concentration of citric acid was found to be ,

MUSHROOM CULTIVATION

Aim:

To know cultivation technology of edible mushrooms cultivation of oyster mushroom spawn production.

Introduction:

Mushrooms are fresh fungi it's constitute a major group of lower plant kingdom. The mushroom is a common fungal fruit body that produces basidiospores at the tip called bacial. The mushroom consisting of short stem and a cap which being to open like an umbrella. India is the second most popular country of the world with a population of over 100 crores. Mushrooms provides a rich addition to the diet in the form of protein, carbohydrates,minerals,and vitamins. Nutrient value of mushroom include 91.1% moisture, 29.9% protein, 4.4% carbohydrate, 0.3%fat and 16k calorie.

Presently about a dozen fungi are cultivated in over 100 countries with production of 2.2 million Agaricus bisporus (56%) , in which button colonies Lentinus edodes (14%) shiltake volvariella vlovacea (8%) paddy straw: pleuritis(7%)- oyster: Flammulina (5.5%) are the mushroom Tremmula(4.6%)- silvered,Phillipa(1.8%) nancelia varieties cultivated for human usage.

Species of the genus pleurotus called oyster mushroom Dhigngri or wood fungus is ranked as the 4th important mushroom of the world amount the 5 most important cultivate mushroom with a production of 15000 tons per annum. The genus contain over 50 species of these P.ostreaus, P.flabellatus, P.saforcaju, P.sapidus, P. Fossulatus have been cultivated in india

OYSTER MUSHROOM CULTIVATION:

SPAWN PRODUCTION:

Successfully cultivation of any mushroom on small scale, one of the most important requirements is the seed. Spawn is the pure culture of the mycelium grown on special medium. It is a mushroom seed, comparable to the vegetative seed in crop plants. Spawn production mainly consist of three steps. They are substrate preparation., Substrate inoculation and incubation of the inoculated substrate.

MATERIALS REQUIRED:

Pure culture of pleurotus, cereal grain, calcium sulphare, calcium carbonate, glucose bottles , cotton, cooker, laminar air flow cabinet, incubator, wine guage, balance, Bunsen burner, water,etc....

PROCEDURE:

Take 600g of grain in 400 to 600 ml of water in a container.

Boil the grain for 15 to 20 mints to bring the moisture content of the grain to 40 to 50%.

Remove the excess water fom grain by spreading the grain in the sieve.

Allow the grain to surface dry by spreading over the dry surface in shade for a few hours.

Mix the grain thoroughly with chemicals,(2% calcium sulphate and 0.5% calcium carbonate) on dry weight basis to adjust the ph of the grain.

Fill the grain- chemical mixture in 500ml glucose bottle/ plastic bag.

Plug the bottle with non absorbent cotton.

Sterile the substrate by autoclaving at 121℃ for 30 minutes.

Repeat the process of sterilization after 24 hrs of 1st autoclaving.

Allow the substrate contents to reach room temperature. Now the substrate is ready for inoculation.

Inoculate the substrate with mycelium of the mushroom growth on a specific medium bt transferring mycelium agar on the grain under aseptic condition.
Shake the containers after plugging to distribute the fragments of the mycelium.
Store the inoculated container at 20 to 25℃ in darkness for three weeks.
Shake the containers for even an distribution of mycelium, after a few days of incubation mycelium is visible on the grain.

OBSERVATION AND RESULT:

When coloured mycelium growth is noted. This indicates seed of mushroom is completely grown and utilized for oyster mushroom production.

OYSTER MUSHROOM CULTIVATION:

The oyster mushroom are rich in protien,minerals contents,devoid and low in calories and carbohydrates. These are ideal food for diabetic and heart patients and those who do not want to put an weight. The various substrates utilized for the cultivation of Pleuritis are banana Pseudonyms,wheat straw,ragi straw,compost prepared from straw,saw dust, sunflower stalks,rich husk and karashay. However the highest yields are obtained on rice straw. There can be grown in any container of earthen pot, cane gasket, polyethylene bags,iron basket or in wooden trays.

MATERIAL REQUIRED:

Thatched hut, polyethylene chamber,mud/Lucca house,dry paddy straw (chopped)or other agro-wastes-100kg,horse gram powder-4kg,spawn bottles of Pleuritus sps, polyethylene bags,1kg, water sprayer.

PROCEDURE:

Take dried paddy straw.
Chop the straw into 1to2 cm bits.
Soak the chopped straw into water over night.
Drain off the excess water.
Add horse gram powder at rate of 8g/kg.
Add spawn at the rate of 30g/kg.
Mix all the constituents.
Fill the mixture into polyethylene bags with holes.
Incubation the field bags in room at 21to35℃ with sufficient light and ventilation for 15 - 16 days for spawn running.
Spray water over the bags twice a day.

OBSERVATION:

Observe for the mushroom crop after 3to4 days of opening the polyethylene bags.

HARVESTING:

First harvesting is to be done 20-22 days after spawning ,2nd harvesting 27-29 days after spawning and 3rd harvesting 34-36 days after spawning.

PRECAUTIONS:

For better yields,temperature ot the room should be in the range of 20-26℃ and relative humidity of 70-90%
Provide light for 15 to 20 minutes during cropping period.
Polyethylene bags with holes should be used.

RESULT:

Oyster mushroom is cultivated and harvested

IMMOBILIZATION TECHNIQUE (SODIUM ALGINATE METHOD)

Aim:

To entrap the live cells in a solid matrix and analysis its activity

Principle:

The Technique involves a reaction between sodium alginate and calcium chloride forming calcium alginate with the optimum concentration of the formation of beads. This property could be used as a suitable technique to immobilize microbial cells in the form of beads. Entrapped in insoulble alginate gel is recognized as a rapid non toxic, inexpensive versatile and the most often used method for immobilization of cells more than 80% of cell immobilization process are still carried out using alginate. Immobilization offers many potential advantages over free cell system such as higher cell densities and cell loads increased smaller fermenter size which may lower capital.The reverse of the same bio catalyst for prolonged period of the time due to constant cell regeneration. A continues process which may be performed beyond the nominal washout rate improved substrate utilization, reduced risk for microbial contamination, improvd substrate utilization, constant product quality & production of wire from substrate and end product inhibition.

Materials Required :

Sodium alginate - 5.5g

Culture: any one type of seed culture for fermentation

Media: PDA, Growth medium, fermentation medium.

Procedure :

1. Preparation of seed culture

*prepare growth medium and sterlize at 110°c for 10 minutes

*inoculate a loopful of culture from slant or 1ml of culture from broth

* incubate at 37°c for 18 hours

2. Preparation of immobilized cells:

* prepare sodium alginate slurry by adding 5.5g of sodium alginate to 150ml of 0.1% Nacl solution with continues slurring

* allow the slurry to stand for 6-8 hours and add 15ml of seed culture

* prepare the beads by controlled drop wire addition of slurry to 4% Cacl2 solution

* keep the beads in Cacl2 solution about 30minutes

3. Activation of immobilized beads:

* perform activation of immobilized beads with help of growth medium

* inoculate required volume of beads into the rich medium and keep it for 30 minutes

* note the movement of beads

4. Fermentation of alcohol using immobilized beads:

* add 25ml of beads to 250ml of fermentation medium and carry out the fermentation under static condition at room temperature

* estimate the alcohol content using through chronic acid methods

* measure volume beads using water displacement method

Observation

*Spherical shaped immobilized beads are prepared and activate in growth medium

Discussion:

The cells are immobilized like the beads in calcium alginate gel. The gel can be solubilized by the solution of phosphate on or citrate or EDTA. Alginate forms gel in the presence of calcium through the formation of ionic bridge