SEMESTER VI

17BTU611B BIOTECHNOLOGY AND HUMAN WELFARE PRACTICAL 3H - 1C

Total hours/week: L:0 T:0 P:3 Marks: Internal: 40 External: 60 Total: 100

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1. Ethanol fermentation using Baker's yeast
2. Study of a plant part infected with a microbe
3. To perform quantitative estimation of residual chlorine in water samples
4. Isolation and analysis of DNA from minimal available biological samples
5. Case studies on Bioethics (any two)

(Wherever wet lab experiments are not possible the principles and concepts can be demonstrated through any other material or medium including videos/virtual labs etc.)

References

1. Sateesh ,M.K. (2010). *Bioethics and Biosafety*. I. K. International Pvt Ltd.
2. Sree Krishna, V. (2007). *Bioethics and Biosafety in Biotechnology*. New age international publishers.

Class: III B.Sc. BT Course Name: BIOTECHNOLOGY AND HUMAN WELFARE PRACTICAL
Course Code: 17BTU611B Batch: 2017

1. ETHANOL FERMENTATION USING BAKER'S YEAST

Aim:

To produce ethanol using Baker's yeast.

Principle:

Fermentation is the anaerobic catabolism of a single chemical compound using a series of redox transformations with the goal of generating ATP by substrate-level phosphorylation. Saccharomyces is one of the most studied organisms in science and the major producer of commercial ethanol. Many microorganisms (micro = small), notably yeasts and bacteria, extract energy from their food (glucose) by fermentation. One of the best-known types of fermentation is alcohol fermentation in which the overall chemical reaction is: $C_6H_{12}O_6$ (glucose) → $2CO_2$ + $2CH_3CH_2OH$ (ethyl alcohol) or, starting from sucrose or maltose, $C_{12}H_{22}O_{11} + H_2O$ → $4CO_2$ + $4CH_3CH_2OH$ (ethyl alcohol) Various fruits, especially grapes, could also be fermented to produce alcoholic beverages. Thus, alcoholic fermentation is the process which is responsible for the production of wine, beer, and other fermented products. It is the toxic nature of ethanol which acts to preserve these brews, and which leads to intoxication upon consumption. In fact, yeasts cannot generally survive in alcohol concentrations in excess of approximately 12 to 14%.

Materials required:

Grapes, yeast culture, sugar etc.

Procedure:

1. Harvesting- This is the most critical stage of the process. The grapes must be harvested when the sugar, acid, phenol and aroma compounds are optimised for the style of wine desired.

2. Crushing - The grapes are removed from the stems and gently crushed to break the skins. Sulfur dioxide is added to the grapes at this stage to prevent oxidation and inhibit

microbial activity. Enzymes may also be added to break down the cell walls and aid the release of juice.

3. Pressing- The juice extraction process depends on the type of wines to be used, but always involves squeezing the berries. After pressing the juice is allowed to stand to separate the solids. If necessary the juice may be clarified by filtration or centrifugation.

4. Fermentation- The juice is inoculated with live yeast, which then carries out the fermentation reaction:

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

This reaction occurs through many intermediary biochemical steps. The process is carried out under a blanket of carbon dioxide as in the presence of oxygen the phenols are oxidised and the sugar and ethanol are converted to carbon dioxide and water.

5. Purification- Unwanted solids, salts and microorganisms are removed through a variety of physical processes, then the wine is bottled and sold.

2. STUDY OF A PLANT PART INFECTED WITH A MICROBES

Introduction

In this section, we will concentrate on diagnosing plant diseases caused by pathogenic microorganisms, primarily fungi, bacteria, and viruses. Nematodes are microscopic "worms" that can also cause plant health problems. The study of pathogens and the diseases they cause is the traditional concern of plant pathologists. The specialized training and techniques needed to work with these microorganisms require most plant pathologists to limit their endeavors to specific host or pathogen-related problems.

Types of Pathogens

To diagnose plant diseases effectively, it is necessary to understand the biology of the microorganisms that cause them: fungi, bacteria, and viruses.

Fungi

About 85% of plant diseases are caused by fungi: multi-celled microorganisms that may be seen without a microscope during certain stages of their life cycles. Fungi have no chlorophyll, and their cell walls are composed of chitin and other polysaccharides instead of cellulose, which composes plant cell walls. Many species of fungi can be identified by the microscopic spores they produce—reproductive structures that aid in dispersal and survival. Some fungi have no spores, such as *Rhizoctonia*, which can be identified microscopically by the very characteristic right angle branches of its fungal threads Wind often disperses many fungal pathogens. Spores can be carried for miles by wind. Splashing water, from rainfall or irrigation, will also move fungal spores from plant to plant. Fungi that live in the soil can move from plant to plant by growing along intermingled roots or out from infested plant debris in the soil. Some fungi (e.g., *Rhizoctonia*) can survive on their own for long periods of time without a host by living in plant debris or soil. Fungi can also be spread by human activity, through movement of already diseased plants, or the use of contaminated gardening tools. While fungi may enter a plant

through its natural openings (e.g., stomates), or through wounds, they can also penetrate directly through the plant's cuticle as well.

Bacteria

Bacteria are one-celled microorganisms that are so small they can be seen only with a powerful light microscope. Most plant pathogenic bacteria do not produce spores. Although some bacteria can survive in the soil in decaying plant material for a time, they usually need a host to survive. Bacteria are dependent on outside agents for dispersal from plant to plant. Splashing water (irrigation, wind-driven rain) is the chief means by which bacteria are disseminated. Another important means of dispersal is through human contact. Many bacterial diseases can be spread simply through the process of touching an infected plant and then touching a healthy plant with hands or pruning tools. Bacteria cannot penetrate the cuticle of plants, but must enter the plant through a wound or natural opening to initiate disease. Special sub-groups of bacteria require an insect host for dispersal and entry into the plant. One such example is the citrus disease Huanglongbing (also known as HLB or citrus greening).

Viruses

Viruses are the smallest of the three pathogens described here and can only be seen with an electron microscope. They are made up of genetic material (RNA or DNA), which is usually wrapped in a protein coat. They must have a living host in order to reproduce, because they use plant host cells in the reproduction process. Most fungi and bacteria reproduce independent of the plant host. Viruses are usually spread from diseased to healthy plants by insects, but can also be spread by mites, nematodes, fungi, and even humans. The organism spreading the virus is referred to as a vector. In Florida, most viruses are vectored by insects, primarily aphids or whiteflies.

Symptoms

Symptoms are abnormal features of the plant that indicate something is wrong. It is important to learn the proper name for a symptom. Many are self-explanatory. A spot is just that, a spot. It is

also necessary to mention the part of the plant exhibiting the symptom. If there are spots on the leaves, they will be called leaf spots; spots on the fruit are fruit spots. The technical term for a spot is "lesion," which means a localized diseased area or wound. As spots grow together (coalesce), the symptom is called a blight. This differs from a spot because larger amounts of tissue are affected. Galls, or tumors, may be found on stems, roots, or sometimes on leaves. These are masses of undifferentiated tissue growth, similar to cancerous tumors in people. They can be easily confused with those caused by insects. Cankers are sunken lesions, which are found most often on stems but can also occur on tree trunks. Wilts and rots are just what the names imply. It is important to note that a rot does not have to be wet and "yucky"; there are dry rots. A rot simply means the plant tissue is being degraded by the pathogen. To tell if a pathogen is responsible for a wilt, make a vertical cut (cross-section) near the base of the plant or individual wilted stem. If a pathogen is present, the vascular (water-conducting) tissue will appear dark. A plant wilting from water stress will have normal white, off-white or light-green vascular tissue.

Preliminary Diagnostic Equipment

As indicated in this discussion, a few simple tools are useful for preliminary diagnosis of plant diseases. These include a hand lens, sharp knife, clear glass container or jar, plastic storage container and rubbing alcohol. A hand lens is often necessary to see the fungal growth on a lesion. The knife is used to make cross-sections of stem tissue. Clean the knife after each use with a tissue or cotton ball soaked with rubbing alcohol. The glass jar is used for the bacterial streaming test, and the storage container becomes a moisture chamber for inducing fungal growth from infected tissue.

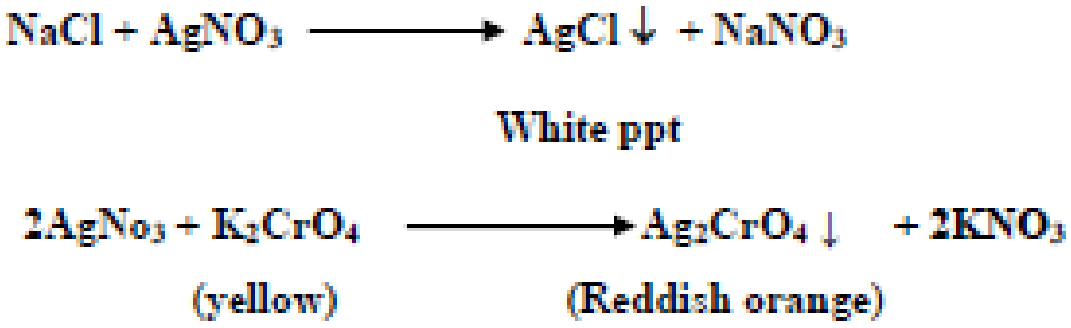
3. QUANTITATIVE ESTIMATION OF RESIDUAL CHLORINE IN WATER SAMPLES

AIM

To determine the amount of residual chlorine present in the given water sample. **PRINCIPLE** Chlorides are usually present in water as NaCl, MgCl2 and CaCl2. If the chloride content of water is greater than 250 ppm, this imparts a peculiar taste to the water and makes the water unfit for drinking purpose. Presence of chlorides is also undesirable in boiler feed water.

Chloride ions present in water can be determined by titration with standard AgN03 solution, using potassium chromate as indicator. This method is called Argentometric method or Mohr's method.

The chloride ions present in the water react with AgNO3 forming insoluble white precipitate of AgCl. As soon as all the chloride ions are removed in the form of AgCl, the extra drop of AgNO3 reacts with the indicator forming red silver chromate. Thus the end point is the change of colour from bright yellow to distinct reddish brown colour.



TITRATION –I STANDARDISATION OF AgNO3 SOLUTION:

Burette solution : AgNO**3** solution

Pipette solution : Standard NaCl solution

Indicator : potassium chromate

End point : appearance of reddish brown colour.

S.No	Volume of Std NaCl solution (ml)	Burette reading (ml)		Volume of AgNO3 (ml)	Concordant value
		Initial	Final		
1	20				
2	20				
3	20				
4	20				

CALCULATION:

Volume of NaCl V1 = 20 ml

Normality of NaCl N1 = 0.01N

Volume of AgNO3 V2 = ml

Normality of AgNO3 N2 = ?

According to volumetric law, V1 N1 = V2N2 N2 = V1 N1/V2

Strength of AgNO3 N2 = …….. N

TITRATION –II

ESTIMATION OF CHLORINE CONTENT IN THE WATER SAMPLE

Burette solution : AgNO3 solution

Pipette solution : sample water

Indicator : potassium chromate

End point : appearance of reddish brown colour.

S.No	Volume of water	Burette reading (ml)	Volume of AgNO3	Concordant

	sample (ml)	Initial	Final	(ml)	value
1	20				
2	20				
3	20				
4	20				

TITRATION –III

ESTIMATION OF CHLORINE CONTENT IN THE WATER SAMPLE

Burette solution : AgNO3 solution

Pipette solution : sample water

Indicator : potassium chromate

End point : appearance of reddish brown colour.

S.No	Volume of distilled water (ml)	Burette reading (ml)		Volume of AgNO3 (ml)	Concordant value
		Initial	Final		
1	20				
2	20				
3	20				
4	20				

PROCEDURE

TITRATION -I

STANDARDISATION OF AgNO3 SOLUTION:

The burette is washed well with distilled water and rinsed with small amount of AgNO3 solution. It is then filled with the same solution upto the zero mark without any air bubbles. The pipette is washed with distilled water and rinsed with the small amount of Standard NaCl solution. 20 ml of NaCl solution is pipette out into a clean conical flask. Add one ml of potassium chromate

indicator. Titrate the solution against AgNO3 solution taken in the burette. End point is the change of colour from yellow to reddish brown. Repeat the titration for the concordant values.

TITRATION-II & III

ESTIMATION OF CHLORINE CONTENT IN THE WATER SAMPLE:

Pipette out 20 ml water sample into a clean conical flask. Add one ml of potassium chromate indicator. Titrate the solution against standardized AgNO3 solution taken in the burette. End point is the change of colour from yellow to reddish brown. Repeat the titration for the concordant values.

RESULTS: The amount of residual chlorine in the given sample of water =

4. ISOLATION AND ANALYSIS OF DNA FROM MINIMAL AVAILABLE BIOLOGICAL SAMPLES

Aim:

To isolate total genomic DNA from the given plant tissue sample **Principle:** High molecular weight DNA free from protein and RNA should be the basic technique for DNA isolation

protocol. The isolated high molecular weight genomic DNA is essential for all molecular biology experiments. The cell wall of plant cell must be broken or digested to release cellular components, which generally performed by grinding with dry ice or liquid nitrogen. The plasma membrane of cell must be disrupted using detergents (SDS or CTAB). The release DNA must be protected from endogenous nucleases using EDTA. The released cellular components generally emulsified with either chloroform or phenol to denature the proteins. The released DNA must be precipitated using isopropanol. The precipitated DNA then washed with alcohol to remove the salt present in the DNA.

Materials required:

1. 2X CTAB Lysis Buffer - containing

    0.1 M Tris HCl (pH 8.0)

    20 mM Na EDTA (pH 8.0)

    1.4 M NaCl

    2% CTAB (W/V)

    2% PVP (W/V)

    0.3% β-mercaptoethanol or Na2S2O5 (add when used).

2. 1X CTAB lysis buffer – dilute 2X CTAB lysis buffer using distilled water (1:1 ratio)

3. Chloroform:isoamyl alcohol (24:1)

4. Ice cold isopropanol

5. 3M sodium acetate (pH 5.2)

6. TE buffer (pH 8.0)

Procedure:

1. 0.1 g of plant tissue was ground using liquid nitrogen to get fine powder.

2. The ground fine powder was the transferred into 2 ml eppendorf tube. (Do not allow the sample to thaw)

3. Immediately, 0.9 ml of CTAB lysis buffer was added and vortexed vigorously.

4. Then incubated at 65ºC for 30 min. The contents were mixed frequently during the incubation.

5. Then centrifuged at 12000 rpm for 5 min, the supernatant was transferred into new 2 ml eppendorf tube.

6. To the pellet, 0.3 ml of CTAB lysis buffer was added, mixed well by vortexing and again incubated at 65ºC for 10 min.

7. The contents were centrifuged at 12000 rpm for 10 min and the supernatant was transferred into the 1st supernatant sample.

8. To the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) was added and invert mixed for 15 min, centrifuged at 10000 rpm for 10 min at room temperature.

10. The aqueous layer was transferred into a new 1.5 ml eppendorf tube.

11. 0.7 volume of ice cold isopropanol and 10 µL of 3M sodium acetate (pH 5.2) was added to the aqueous layer, the contents were mixed by invert mixing.

13. Then centrifuged at 10000 rpm for 10 min at room temperature.

14. The supernatant was discarded, and 70 % ethanol was added to the DNA pellet.

15. Again centrifuged at 10000 rpm for 10 min. 16. Then the ethanol supernatant was discarded and the pellet was subjected to air drying.

17. After air drying, the pellet was resuspended in 50 μL TE buffer (pH 8.0).

18. After DNA has dissolved, the purity of the DNA was checked by electrophoresis and spectrophotometric analysis.

19. 0.8 % agarose gel was prepared using 1X TAE buffer.

20. Isolated DNA samples were loaded into the wells, recording which samples are loaded into which wells as lane 1, 2, etc. The power supply was started after sample loading, with the voltage set to 50 V.

21. Ran the gel until the second dye from the well has reached 3/4 th of the gel.

22. After the run was completed, the gel was observed under UV transilluminater.

24. Then DNA was stored at 4°C short term, –20°C or –80°C long term.

5. CASE STUDIES ON BIOETHICS

Bioethics is the study of the ethical issues emerging from advances in biology and medicine. It is also moral discernment as it relates to medical policy and practice. Bioethicists are concerned with the ethical questions that arise in the relationships among life sciences, biotechnology, medicine, politics, law, and philosophy. It includes the study of values ("the ethics of the ordinary") relating to primary care and other branches of medicine.

Purpose and scope

The field of bioethics has addressed a broad area of human inquiry, ranging from debates over the boundaries of life (e.g. abortion, euthanasia), surrogacy, the allocation of scarce health care resources (e.g. organ donation, health care rationing) to the right to refuse medical care for religious or cultural reasons. Bioethicists often disagree among themselves over the precise limits of their discipline, debating whether the field should concern itself with the ethical evaluation of all questions involving biology and medicine, or only a subset of these questions. Some bioethicists would narrow ethical evaluation only to the morality of medical treatments or technological innovations, and the timing of medical treatment of humans. Others would broaden the scope of ethical evaluation to include the morality of all actions that might help or harm organisms capable of feeling fear.

The scope of bioethics can expand with biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics and life in space, and manipulation of basic biology through altered DNA, RNA and proteins. These developments will affect future evolution, and may require new principles that address life at its core, such as biotic ethics that values life itself at its basic biological processes and structures, and seeks their propagation.