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Faculty of Science

Laboratory Manual

Fermentation Technology

Bachelor of Biotechnology (Hons.)

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Fermentation Technology

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LINCOLN UNIVERSITY COLLEGE
FACULTY OF SCIENCE (DEPARTMENT OF BIOTECHNOLOGY)
LABORATORY SAFETY RULES

The following rules must be obeyed by all students in the science laboratory of the faculty. Wilful or repeated in advertent non-compliance may result in dismissal or suspension from the laboratories

- No entry without permission:
  - Outsiders are not allowed to enter the laboratory without permission.
  - No student is allowed to enter the laboratory unless permission has been given by a laboratory assistant or a lecturer.

- At work in the laboratory:
  - No experiment may be attempted without the knowledge and permission of a lecturer.
  - Students must wear shoes in the laboratory. Students wearing slippers or sandals are not allowed to work in the laboratory.
  - Lab coat must be worn at all times during practical work in the laboratory.
  - Do not mouth pipette chemicals.
  - Do not eat or smoke in the laboratory.
  - Do not taste any chemicals, including dilute solutions. If any acid or alkali accidentally enters your eyes or mouth, wash immediately with plenty of tap water. Inform your lecturer, and seek medical attention if necessary.
  - Paper should be used to light up the Bunsen burners.
  - Used match sticks, filter papers, and other solid waste must never be thrown into the sinks. They must be thrown into the dustbins provided. Lighted match sticks and smoldering materials must be extinguished with tap water before thrown in to the dustbins.
  - Any equipment broken or damaged must be reported to the laboratory assistant.

- Before leaving the laboratory:
  - All the equipment and benches must be cleaned at the end of each practical session.
  - Wash hands and arms with soap and water before leaving the laboratory.
  - No student is allowed to take away any chemicals, equipment or other property of the laboratory.

INTRODUCTION

1. The Scientific Method

- Making observations
- Generating hypotheses
- Making predictions
- Designing and carrying out experiments
- Constructing scientific models

2. Practical Exercises

To get the most out of the practical exercises, you need to follow carefully the instructions given. These instructions have been designed to provide you with the experience in the following skills:

- Following instructors
- Handling apparatus
- Having due regard for safely
- Making accurate observations
- Recording results in an appropriate form
- Presenting quantitative results
- Drawing conclusions

3. Following Instructions

Instructions are provided in the order in which you need to carry them out. We would advise that before carrying out the instructions, you read through the entire exercise. This will help you to remember what you have learned.

Each practical exercise in the book begins with a few lines describing its purpose in most cases the following headings are also used:

- Procedure-numbered steps that need to be carried out.
- For consideration -some questions to help you think carefully about the results you have obtained.
- Materials-a list of the apparatus, chemicals and biological materials you need.

4. Handling apparatus

Biologists need to able to use many different types of apparatus, for example, photometers (to measure water uptake by plants), respirometers (to measure oxygen uptake or carbon dioxide production), Petri dishes (for plating out bacteria and other microorganisms) and the light microscope (to magnify specimens). Many of the practical exercises are designed to help you derive the maximum benefit from a piece of apparatus.

5. Having Due Regard for Safety

Surveys have been shown that science laboratories are among the safest places to be. Nevertheless, this is no cause for complacency.

- Always move slowly and carefully in a laboratory.
- Never put your fingers in your mouth or eyes after using chemicals or touching biological specimens until you have washed your hands thoroughly with soap and warm water, and dried them.
- Make sure glass objects (e.g, thermometers, beakers) cannot roll off tables or be knocked onto the floor.
- Wear safely goggles whenever there is a risk of damage to the eyes.

<u>Situations of risk include:</u>

- Heating anything with a Bunsen burner (even heating water has its dangers')
- Handling many liquids, particularly those identified as corrosive, irritant, toxic or harmful

- Handling corrosive or irritant solids
- Some dissection work
- Allow Bunsen burners, tripods, gauzez and beakers to cool down before handling them.
- Never allow your own body fluids (especially blood and saliva) to come into contact with someone else, or theirs into contact with you.
- Keep long hair tied back and do not wear dangly earrings.
- Do not allow electrical equipment to come into contact with water.
- If you are unsure how to carry out a scientific procedure, ask.
- Make sure you understand why you are going to do something before you do it.
- Wear a lab coat when using chemicals or handling any biological specimens.
- Follow exactly agreed procedures with regard to cuts, burns, electric shocks and other accidents (e.g. with chemicals).
- Follow exactly all specific safely instructions given in this book or provided by your teacher for particular practical exercises (e.g. use of gloves, disinfection)

With practice, these procedures should become second nature to you. They will enable you to carry out practical work in safety.

6. Making Accurate Observations

In most cases the practical exercise will make it clear what you need to observe, e.g. the time taken for a certain volume of gas to be evolved or the width of a sample cells. Ensure that you know how to use any necessary equipment before starting practical. Think carefully about the precision with which you will make your observations.

7. Recording Results in an Appropriate Form

Results can be recorded in various ways. Often it is helpful to record raw data in a table. Most data will be in the form of numbers, i.e. they will be quantitative data (also known as numerical data). However, some data, e.g. flower colour, will be qualitative data.

One form in which some biological findings can be recorded is a drawing. You don't need to be professional artist to make worthwhile biological drawings. If you follow the following guidelines, a drawing can be of considerable biological value:

- Ensure that your completed drawing will cover at least a third of A4 page.
- Plan your drawing so that the various parts are is proportion and will not be drawn too small. Small marks to indicate the length and breadth of the drawing are a great help in planning and a faint outline can be rapidly drawn to show the relative positions of the parts.
- The final drawing should be made with clean, firm lines using a sharp HB pencil and, if needed, a good quality eraser (not a fluid).If important details are too small to be shown in proportion, they can be put in an enlarged drawing at the side of the main drawing.
- Avoid shading and the use of colour unless you are an excellent artist and they really help, for example when drawing soil profiles.
- When drawing structures seen with the naked eye or hand lens, use two lines to delineate such things as blood vessels and petioles. This will help you to indicate the relative widths of such structures.
- When drawing low power plan drawings from the light microscope, do not attempt to draw individual cells-just different tissues.
- When drawing plant cells at high power under the light microscope, use two lines to indicate the width of cell walls, but a single line to indicate a membrane.
- Always put a scale on each drawing.

8. Presenting Quantitative Results

Presentation of data is all about using graphs or other visual means to make it easier to see what your results tell you. The following four ways of presenting data are the most frequently used in biology: line graphs, bar charts, histograms and scatter graphs (Figure 1).

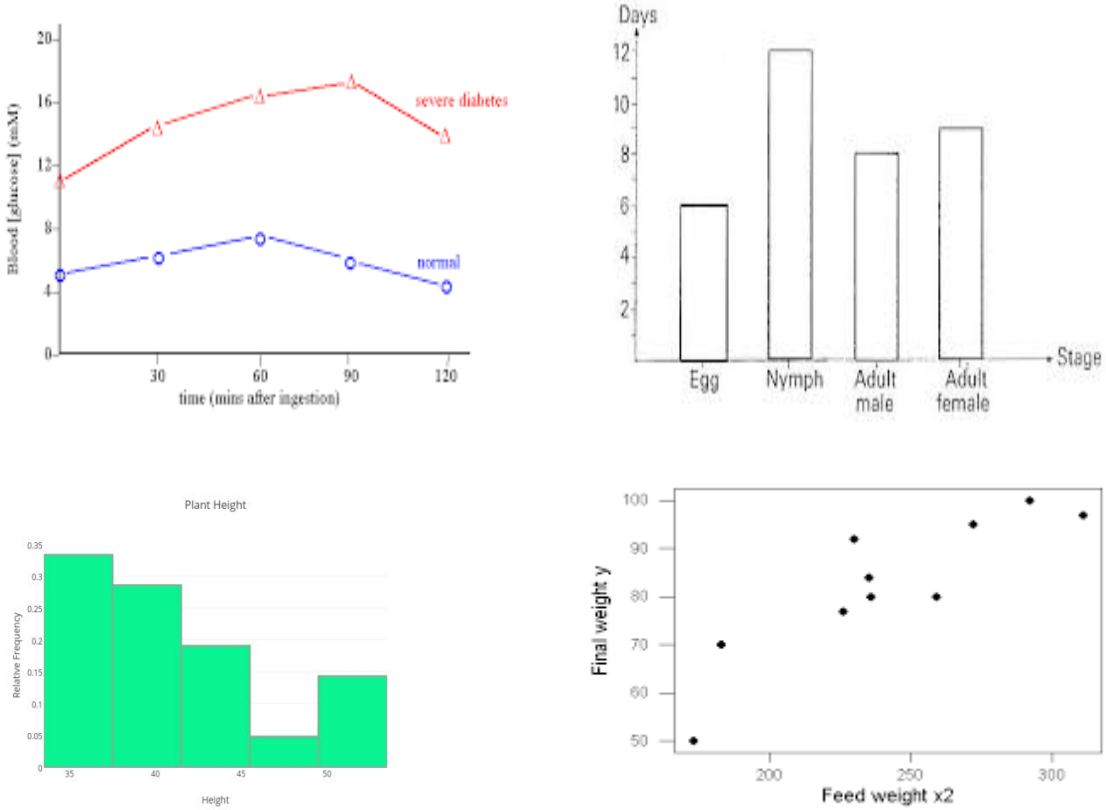


Figure 1: Line graphs, bar charts, histograms and scatter graphs

9. Drawing Conclusions

Finally, you will need to draw conclusions. If your practical exercise has involved the testing of a hypothesis, for example that the enzyme pepsin works better at low pH than in neutral or alkaline conditions, your conclusion should indicate whether the hypothesis has been refuted (i.e. shown not to be the case) or supported. Of course, even if your hypothesis has been supported, it doesn't mean that it has been confirmed with 100% certainty- in other words it isn't proved. Science proceeds more by showing that certain ideas are wrong than by showing that others are right (think about that!). Your conclusion might therefore include further ways of testing the original hypothesis, or might raise new possibilities to be investigated.

Often you will only be able to arrive at your conclusions after statistically analysing your data.

10. Writing a Scientific Lab Report

Title

- Communicate the subject investigated in the paper.

Introduction

- State the hypothesis.
- Give well-defined reasons for making the hypothesis.
- Explain the biological basis of the experiment.
- Cite sources to substantiate background information.

- Explain how the method used will produce information relevant to your hypothesis.
- State a prediction based on your hypothesis. (If the hypotheis is supported, then the results will be.)

Materials and Methods

- Use the appropriate style.
- Give enough detail so the reader could duplicate your experiment
- State the control treatment, replication and standardized variables that were used.

Results

- Summarize the data (do not include raw data).
- Present the data in an appropriate format (table or graph).
- Present tables and figures neatly so they are easily read.
- Label the axes of each graph completely.
- Give units of measurement where appropriate.
- Write a descriptive caption for each table and figure.
- Include a short paragraph pointing out important results but do not interpret the data.

Discussion

- State whether the hypothesis was supported or proven false by the results, or else state that the results were inconclusive.
- Cite specific results that support your conclusions.
- Give the reasoning for your conclusions.
- Demonstrate that you understand the biological meaning of your results.
- Compare the results, with your predictions and explain any unexpected results.
- Compare the results to other research or information available to you.
- Discuss any weaknesses in your experimental design or problems with the execution of the experiment.
- Discuss how you might extend or improve your experiment.

Conclusion

- Restate your conclusion.
- Restate important results.

Literature Cited

- Use the proper citation form in the text.
- Use proper citation form in the Literature Cited section.
- Refer in the text to any source listed in this section.

Acknowledgement

- State any appropriate acknowledgement that you think is necessary.

Practical 1
Title: Isolation techniques of microbes

Objective:
After completing the practical, you will be able:

1. To isolate microbes from soil

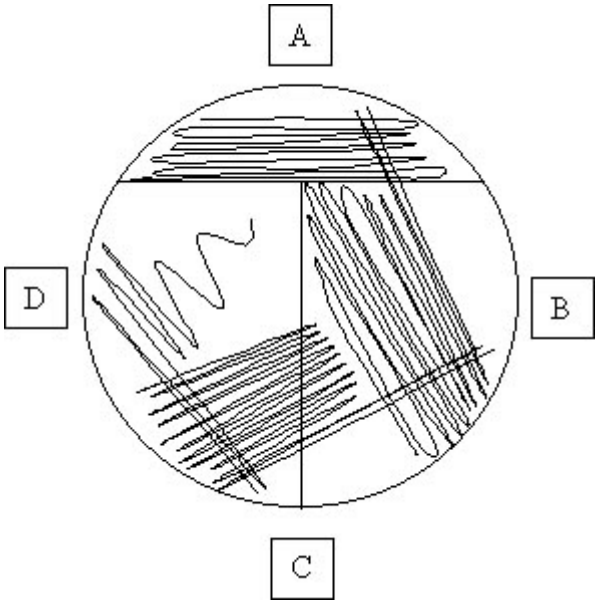
Introduction

Microorganisms occur in a natural environment like soil, water and air (Lecomte, St-Arnaud, & Hijri, 2011). They interact and mix with several other forms of life. Many microbes are pathogenic causing a number of diseases with a variety of symptoms, depending on how they interact with the patient. The isolation and growth of suspected microbe in pure culture is essential for the identification and control of the infectious agent.

The primary culture from natural source will normally be a mixed culture containing microbes of different kinds. However, in the laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species is known as isolation of the organisms. The isolation of bacteria forms a very significant step in the diagnosis and management of the illness. Isolation of bacteria involves certain steps such as specimen collection, preservation, and transportation of specimen as well as microscopic examination of the sample.

Common techniques for isolating and culturing microbes are discussed below:

Streak plate method: The loop is used for preparing a streak plate. This involves the progressive dilution of an inoculum of bacteria or yeast over the surface of solidified agar medium in a Petri dish in such a way that colonies grow well separated from each other. The aim of the procedure is to obtain single isolated pure colonies.



Pour plate method:

Pour plate is one in which a small amount of inoculum from broth culture is added by pipette to the center of a Petri dish. Molten, cooled agar medium in a test tube or bottle, is then poured into the Petri dish containing the inoculum. The dish is gently rotated to ensure that the culture and medium are thoroughly mixed and the medium covers the plate evenly. Pour plates allow micro-organisms to grow both on the surface and within the medium. Most of the colonies grow within the medium and are small in size and may be confluent; the few that grow on the surface are of the same size and appearance as those on a streak plate. If the dilution and volume of the inoculum, usually 1 cm^3, are known, the viable count of the sample, i.e. the number of bacteria or clumps of bacteria, per cm3 can be determined. The dilutions chosen must be appropriate to produce between 30 and 100 separate countable colonies.

Spread plate method:

Spread plates, also known as lawn plates, should result in a heavy, often confluent growth of culture spread evenly over the surface of the growth medium. This means that they can be used to test the sensitivity of bacteria to many antimicrobial substances, for example, mouthwashes, garlic, disinfectants, and antibiotics. The spread plate can be used for quantitative work (colony counts). If the dilution and volume of the inoculum, usually 0.1 cm^3, are known, the viable count of the sample, i.e. the number of bacteria or clumps of bacteria per cm^3, can be determined. The dilutions chosen must be appropriate to produce between 30 and 100 separate countable colonies.

Materials

- Spatula
- Cylindrical bottles
- Vortex
- Pipette
- Petri dish
- Agar
- Incubator
- Colony counter

Procedure:

1. Preparation of soil dilutions

1. Weigh out 10 g of soil sample
2. Add about 90 - 95 ml of deionized water to the soil sample. Shake the suspension well, and label as “A”.
3. Use a sterile pipette to take 1 ml of the suspension and transfer it to a 9 ml deionized water blank. Vortex thoroughly, and label as “B”.
4. Repeat this dilution step three times, each time with 1 ml of the previous suspension and a 9-ml deionized water blank. Label these sequentially as tubes C, D, and E. This results in serial dilutions of 10^{-1} through 10^{-5} grams of soil per ml.

2. Making spread and pour plates for bacterial culture

1. To grow bacterial colonies, take three pre-prepared agar plates (from different agar) and labels them as C, D, and E. Vortex samples C, D, and E, and pipette 0.1 ml onto each plate. This increases the dilution value further, by a factor of ten (C = 10^{-3}, D = 10^{-4}, E = 10^{-5}).
2. Next, dip a glass spreader into ethanol. Place the spreader in a flame for a few seconds to ignite and burn off the ethanol. This will sterilize the spreader.
3. Hold the spreader above the first plate until the flame is extinguished. Open the plate quickly, holding the lid close by. Touch the spreader to the agar away from the inoculum (Inoculum = cells used to begin a culture) to cool, and then spread the drop of inoculum around the surface of the agar until traces of free liquid disappear. Replace the plate lid.
4. Re-flame the spreader and repeat the process with the next plate, working quickly so as not to contaminate the agar with airborne organisms.
5. Pipette 0.1 mL from step 1 above for pour plate procedure.
6. Incubate the bacteria plates inside the incubator at 37 ºC for 48 hours. Make sure the plates are inverted during the incubation to prevent drops of moisture from condensation from falling onto the agar surface.

3. Bacterial counts

1. After incubation, examine all of the bacteria plates carefully and note differences in colony size and shape. When grown on agar, bacteria produce slimy colonies ranging from colorless to bright orange, yellow, or pink. In contrast, actinomycete colonies are chalky, firm, leathery, and will break under pressure, where other bacterial colonies will smear. This allows colonies to be distinguished by touch with a sterile loop.
2. Count and record the number of bacterial colonies, including any actinomycetes. Only count plates with 30-200 colonies per plate.

4. Making Spread Plates for bacterial culture

1. To obtain single isolated pure colonies, use sterile loop to pick a single colony from any of the plates
2. Streak it on a new agar plate

Report the results

1. Use the above steps to determine the result as colony-forming units per mL of sample (CFU/ml).
2. Use the colony counter to look at the colonies on the agar plate. Make an estimate of the number of colonies in each square of the grid.

Note: Make estimated counts only when there are isolated colonies without spreaders. When there are plates with 30 to 300 colonies on each plate, use only those plates to determine the count.

3. Determine the CFU/mL for the estimated colony count.
4. Report the result as CFU/mL. Include in the report the method used, the incubation temperature, time and the nutritional medium. *Example: 75 CFU/mL, pour plate method, 35 °C (95 °F), 48 hours, plate count agar.*

Results:

For pour plate and spread plate, report your observation in the table below:

Plate	No of colony (CFU)	Method used	Incubation temperature	Incubation time	Nutritional medium
C					
C					
C					
D					
D					
D					
E					
E					
E					

Place the photographs of the streak plate

Questions:

1. Differentiate between the pour plate and spread plate method.

2. What is aseptic technique?

Practical 2

Title: Formulation of culture media for fermentation process

Objective:

After completing the practical, you will be able:

1. To learn about composition and types of culture media and how different types of media can be used to study the properties of bacteria

Introduction

The study of microorganisms requires techniques for isolating cells from natural sources and growing them in the laboratory on synthetic media. In other words, the development of synthetic culture media and culture techniques play important roles in the advancement of fermentation and industrial microbiology among others. Generally, bacterial culture media are used for many purposes and applications such as for isolating and identifying bacteria, revealing bacteria metabolic properties, and allowing long-term storage of pure cultures (Hendriks, van Lier, & de Kreuk, 2017).

Taxonomic descriptions of bacteria commonly include information about their cultural requirements; species that are poorly characterized are frequently those most difficult to culture under laboratory conditions. Indeed, Koch's second postulate requires culturing of a suspected pathogen in pure form. Microbiologists traditionally mixed media by combining the individual components listed for a recipe. For a complicated medium, this could be a very time-consuming process. Today, most media are available commercially in premixed and dehydrated form. The media are prepared by simply dissolving the powder in water, sterilizing the solution, and then dispensing it into culture vessels.

Solidified media

Culture media is often prepared in a solid form. Generally, culture media is solidified with the addition of agar, a purified carbohydrate obtained from marine seaweed. Agar is the most widely used solidifying agent because very few bacteria can metabolize it in addition to its unusual property of melting at 100ºC but not resolidifying until the temperature decreases to 45ºC.

Defined media:

Many species of bacteria are identical in appearance and can only be distinguished by their biochemical or metabolic properties. One such property is their nutritional requirements. While all bacteria require similar essential mineral nutrients, such as carbon and nitrogen, species must obtain these nutrients in particular chemical forms. For example, some species can obtain nitrogen from nitrite (NO_2) but not ammonia (NH_3), vice versa, either, or neither.

Defined media contain specific quantities of known substances are commonly used to determine the specific nutritional requirements and capabilities of bacteria. Since defined media typically provide the 'minimal required' source of nutrients, even generalist bacteria may grow more slowly than on complex type of media.

Differential & selective media:

There are many media available that visually distinguish bacteria with particular metabolic characteristics and many media that will selectively grow only certain types of bacteria. One of the most widely used media in clinical settings is MacConkey Agar which has both differential and selective properties. MacConkey agar medium is primarily used to differentiate between Gram-negative enteric Bacteria, such as from stool samples while inhibiting the growth of most Gram-positive bacteria. The medium differentiates bacteria that can ferment lactose, which includes fecal coliform and a variety of

potential pathogens. The selective property of the medium comes from the presence of bile salts and crystal violet that inhibit most Gram-positive bacteria. Fermentation of lactose is differentiated by the neutral red, a pH indicator. When lactose is fermented, acid products lower pH below 6.8, neutral red causes the medium to turn pinkish-red. Colonies of lactose-nonfermenting bacteria will be colorless.

Materials:

- Tryptic Soy Agar (TSA)
- MacConkey agar
- Nutrient agar
- Mueller-Hinton agar
- Spatula
- Pipette
- Petri dish
- Agar
- Incubator

Procedure:

1. In this part of the lab exercise, you will prepare and sterilize different media such as Tryptic Soy Agar (TSA), MacConkey agar, nutrient agar and Mueller-Hinton agar.
2. Prepare each of the agar media according to the manufacturers' instruction.
3. Pipette 0.1 mL of 10^{-5} soil dilution from experiment 1 using using pour plate procedure.
3. Incubate the bacteria plates inside the incubator at 37 ºC for 48 hours. Make sure the plates are inverted during the incubation to prevent drops of moisture from condensation from falling onto the agar surface.
4. After incubation, examine all of the bacteria plates carefully and note differences in colony size and shape.
5. Count and record the number of bacterial colonies.

Results:

Place the photographs of some relevant plates from your work:

Record the number of bacterial colonies

Plate	No of colony (CFU)	Method used	Incubation temperature	Incubation time	Nutritional medium

Questions:

1. How can you identify bacterial growth?
2. What is the purpose of agar in the media?

Practical 3
Title: Laboratory scale fermenter (benchtop fermenter)

Objective:
After completing the practical, you will be able:

1. To introduce the laboratory scale fermenter (benchtop fermenter) to the students

Introduction

Fermenters are the reactors modified and designed for the operation and maintenance of fermentation process using microbial organisms. The vessel used for the fermentation process is generally of cylindrical shape with a round bottom and a round top made up of stainless steel at the pilot and industrial scale. In case of laboratory benchtop fermentors, the body of the vessel is ideally made up of transparent glass of appropriate thickness with a stainless steel top accommodating the provisions for the monitoring of process parameters such as temperature, pressure, dissolved oxygen concentration, pH, antifoam agents etc. and maintenance of sterility on the fermenter.

The process parameters are usually monitored through probes inserted from the top stainless steel plate (in case of benchtop fermenter) and these probes are in turn connected to an electronic control panel where the potentiometric signals of the probes converted in to digital signals for the display during the process for real-time monitoring (Pleissner *et al.,* 2016). Figure 1 shows the picture of fermenter.

Figure 1: Fermenter

In addition, the fermenter is also connected with an air sparger through which the sterile air is spurged into the fermenter. This sterile air is generated using a compressor which sucks the air from the atmosphere and pumps it through an air filter, and this sterile air pipeline is connected to a flow regulation unit, where the air flow rate can be regulated through a knob fitted to the rotameter.

For the regulation of the temperature, a chilled water supply unit is connected to the fermenter externally through a rectangular coil, and the control is provided by the flow control unit as it also

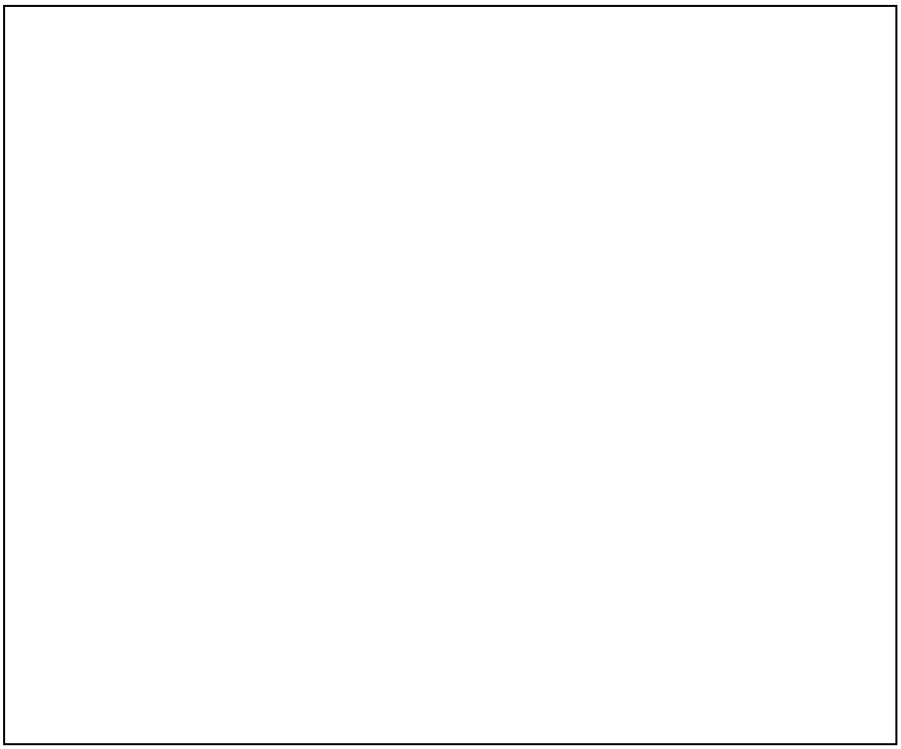
connects the chilled water supply unit and the fermenter. And these all parameters are controlled automatically through the set values of process parameters in the control panel.

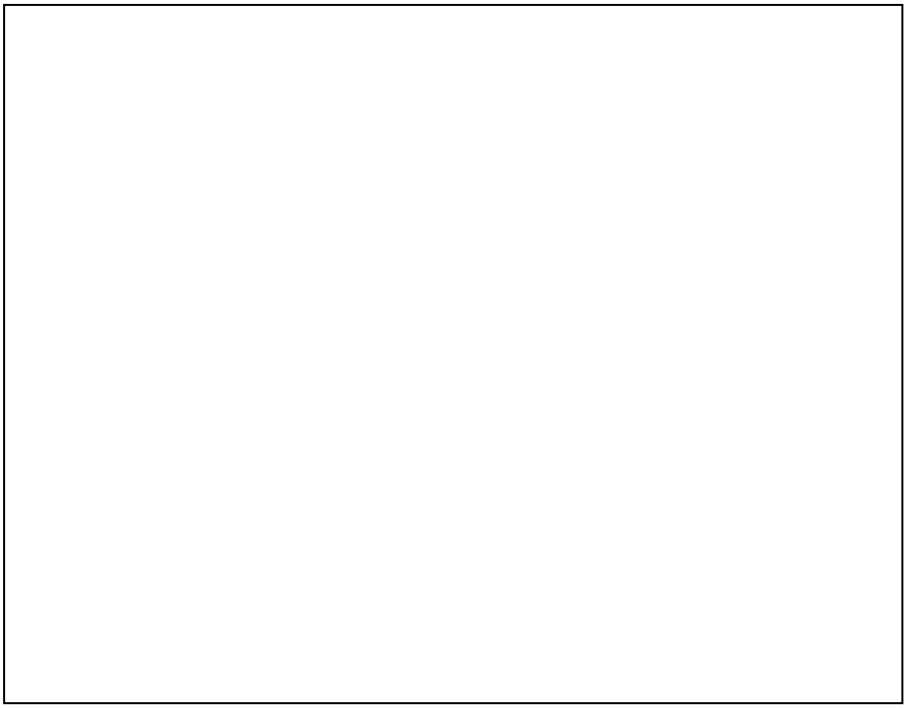
Materials:

- Benchtop fermenter
- Buffering bottles (Acid /Base)
- Peristatic pumps

Procedure:

1. Take a lab note book and a pen with you to the bench top fermenter.
2. With the help of the instructor identify the major parts of the fermenter and familiarize yourself with the equipment.
3. Note all the probes present in the fermenter and identify the connections with the fermenter and control panel.
4. Draw and Label the fermenter unit (Ask the instructor for any clarification and suggestions):
   a- the whole part
   b- the upper plate
6. Write briefly about the major parts of the fermenter in your observations.





Question:

1. Briefly describe the function of the essential parts of a bioreactor.

Practical 4

Title: Estimation of protein concentration using Lowry method

Objective:

After completing the practical, you will be able:

1. To estimate the concentration of protein from different samples using Lowry method

Introduction:

All cells contain hundreds of different biomolecules, including proteins, carbohydrates, lipids, and nucleic acids. These terms refer to classes of compounds and there are actually many types of proteins, carbohydrates, etc. The total amounts of these different molecules vary from cell to cell or from tissue to tissue. An initial step that is often done to characterize a particular cell type is to determine the total amounts of the different types of biomolecules per cell. This is usually accomplished by extracting the molecules from a collection or set of cells and then by doing a spectrophotometric assay to measure the total amount of a certain type of molecule quantitatively. The most accurate method of determining protein concentration is probably acid hydrolysis followed by amino acid analysis (Burtis, Ashwood, & Bruns, 2012).

Most other methods are sensitive to the amino acid composition of the protein, and absolute concentrations cannot be obtained (Waterborg & Matthews, 1996). Colorimetric methods are still frequently used for the estimation of protein concentration as well as a relative comparison between treatments as they are rapid and affordable, do not require a hydrolysis step, and frequently show good correlation to more expensive and time-consuming techniques. The most common colorimetric methods are Bradford assay and Lowry method (Redmile-Gordon, Armenise, White, Hirsch, & Goulding, 2013).

Equipment:

- Pipette
- Pipette tips
- Eppendorf tubes and holders
- Disposable UV-Vis cuvettes - 5 ml
- Spectrophotometer

The blue color developed by the reduction of the Phosphomolybdic-Phosphotungstic components in the Folin-ciocalteau reagent by the amino acids Tyrosine and Tryptophan present in the protein plus the color developed by the biuret reaction of the protein with the color developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry method.

Reagents for Lowry method:

- Lowry Reagent (or) alkaline copper sulfate solution: Mix 50 ml of solution A with 1 ml of solution B, just prior to use.
    - Solution A: 2% sodium carbonate in 0.1 N NaOH
    - Solution B: 0.5% copper sulfate solution in 1% sodium potassium tartarate solution (to be prepared fresh)
- Folin-Ciocalteau reagent: This is commercially available and has to be diluted with equal volume of water just before use.

- Standard protein solution: Dissolve 200 mg of bovine serum albumin (BSA) in 100 ml of distilled water in a volumetric flask. (concentration-2mg/ml).
- Working standard: Dilute 10 ml of stock standard solution to 100 ml of distilled water. (Concentration -200 mg/ml).

Procedure:

1. Pipette out into a series of tubes 0.2, 0.4, 0.6, 0.8, and 1.0 of the protein solution.
2. Make up the total volume to 1 ml with the addition of water.
3. Add to each tube 5 ml of the alkaline-copper sulfate solution.
4. Mix well and allow to standard at room temperature for 10 to 15 minutes.
5. Add 0.5 ml of the reagent into each tube, mixing rapidly after each addition.
6. Leave the tubes for 30 minutes until a blue color is formed.
7. Measure the absorbance with the spectrophotometer at 700 nm.
8. Prepare a blank with 1 ml of distilled water, instead of protein solution and with 1 ml of unknown solution and proceeds as per standards.
9. Prepare a calibration curve with mg of protein on X-axis and O.D. on Y-axis
10. Determine the amount of protein present in given unknown samples.

Report: The concentration of protein in the given unknown samples are:

1. _____ mg/ml
2. _____ mg/ml
3. _____ mg/ml

Question:

1. Briefly describe the procedure and analysis of protein quantification using Bradford reagent.

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Notes

Notes

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