Semester – II

17MBU202 MICROBIAL PHYSIOLOGY AND METABOLISM (4H – 4C)

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

End Semester Exam: 3 Hours

SCOPE

The students will be able to understand and predict the intermediate metabolism of any microbe used in Industrial production processes. This paper also enables the students about to know about microbial nutrition and growth.

OBJECTIVES

- It Gives brief description on the microbial metabolism and its energetics
- It deals with the various aerobic and anaerobic processes through which the organisms obtain and utilize the energy for their growth.
- Explains photosynthesis and photosynthetic bacteria.

Unit I

Microbial nutrition–nutrient requirements, Nutritional groups of microorganisms. Uptake of nutrients by cell – Passive, Facilitated diffusion, Active transport, Group translocation and Iron uptake.

Unit II

Different phases of growth curve - generation time. Measurement of microbial growth. Batch, Continuous and Synchronous culture, Diauxic growth, Influence of environmental factors on growth (Temperature, pH, solute, water activity, oxygen and pressure).

Unit III

Carbohydrate metabolism – EMP, ED, Pentose phosphate pathway, TCA cycle, Aerobic respiration, oxidative phosphorylation, electron transport chain (Prokaryotic and Eukaryotic), substrate level phosphorylation. Anaerobic respiration. Uncouplers and inhibitors.

Unit IV

Anaerobic respiration with special reference to dissimilatory nitrate reduction (Denitrification; nitrate/nitrite and nitrate/ammonia respiration; fermentative nitrate reduction). Fermentation - Alcohol fermentation and Pasteur effect; Lactate fermentation (homofermentative and heterofermentative pathways), concept of linear and branched fermentation pathways

Unit V

Photosynthesis – bacteria and cyanobacteria, photosynthetic pigments – oxygenic (cyanobacterial) and Anoxygenic (Purple, green bacteria) photosynthesis. Nitrogen metabolism-overview of nitrogen cycle.

SUGGESTED READINGS

1. Madigan, M.T., and Martinko, J.M. (2014). Brock Biology of Microorganisms. 14th edition. Prentice Hall International Inc.
2. Moat, A.G., and Foster, J.W. (2002). Microbial Physiology. 4th edition. John Wiley & Sons.
3. Reddy, S.R., and Reddy, M. (2005). Microbial Physiology. Scientific Publishers India.
4. Gottschalk, G. (1986). Bacterial Metabolism. 2nd edition. Springer Verlag.
5. Stanier, R.Y., Ingrahm, J.I., Wheelis, M.L., and Painter, P.R. (1987). General Microbiology. 5th edition, McMillan Press.
6. Willey, J.M., Sherwood, L.M., and Woolverton, C.J. (2013). Prescott's Microbiology. 9th edition. McGraw Hill Higher Education.

Semester – II

17MBU212 MICROBIAL PHYSIOLOGY AND METABOLISM – PRACTICAL (3H – 2C)

Instruction Hours / week: L: 0 T: 0 P: 3 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 6 Hours

SCOPE

The students will get the knowledge of microbial physiology and characterization of the microoganisms.

OBJECTIVES

- To enhance the students knowledge on various aspects of microbial physiology like growth, extremophiles studies and chemical characterization of microbes.

EXPERIMENTS

1. Study and plot the growth curve of *E. coli* by turbidometric and standard plate count methods.
2. Calculations of generation time and specific growth rate of bacteria from the graph plotted with the given data
3. Effect of temperature on growth of *E. coli*
4. Effect of pH on growth of *E. coli*
5. Effect of carbon and nitrogen sources on growth of *E. coli*
6. Effect of salt on growth of *E. coli*
7. Demonstration of alcoholic fermentation
8. Demonstration of the thermal death time and decimal reduction time of *E. coli.*

SUGGESTED READINGS

1. Madigan, M.T., and Martinko, J.M. (2014). Brock Biology of Microorganisms. 14th edition. Prentice Hall International Inc.
2. Moat, A.G., and Foster,J.W. (2002). Microbial Physiology. 4th edition. John Wiley & Sons
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4. Gottschalk, G. (1986). Bacterial Metabolism. 2nd edition. Springer Verlag
5. Stanier, R.Y, Ingrahm, J.I., Wheelis, M.L., and Painter, P.R. (1987). General Microbiology. 5th edition, McMillan Press.
6. Willey, J.M., Sherwood, L.M., and Woolverton, C.J. (2013). Prescott's Microbiology. 9th edition. McGraw Hill Higher Education.

Study and plot the growth curve of *E. coli* by torbidometric and standard plate count method

Aim

To enumerate the cell number of the given microbial sample using turbidometric method and standard plate count.

Principle

An increase in number of cells in a given amount of suspension in a unit time amount of suspension in a unit time signifies growth. Change in cell number in bacterial suspension is measured by incubating the suspension over a period of time and number of cells per ml is measured after every hour. In turbidometric method the cell concentration in a bacterial suspension is measured a s afunction of light transmitted through the suspension and the results is expressed in terms of optical density (OD).

Materials required

Spectrophotometer, yeast growth medium, conical flask, E. coli, pipettes, cuvettes, etc.

Procedure

- The spectrometer was switched on and the wavelength was adjusted at 820nm.
- An overnight culture of E. coli was prepared.
- 1ml of overnight culture was inoculated into a conical flask containing 100 ml of yeast extract broth.
- A cuvette was filled with yeast extract broth (blank) and inserted into the sample holder to adjust the optical density (OD) to zero.
- The yeast cell suspension sample was transferred into a cuvette immediately after inoculation and the initial OD was taken.
- After transferring a small amount of the sample the conical flask was incubated immediately in an orbital shaking incubator at 30 C to ensure aeration and uniform distribution of the cells.
- The OD value of the sample was measured at regular intervals of one hour after inoculation.
- The procedure was repeated and the cells were counted at regular intervals of one hour after inoculation.
- The number of cells per ml of the cell suspension was calculated mathematically as follows

No. of cells/ml = Average number of cells in one large square x 10^4 /cm^3 ie., A+B+C+D+E x 10^4/cm^3

Calculation of specific growth rate and generation time of bacteria

Aim:

To calculate the specific growth rate and generation time of bacteria using graphical method.

Principle:

Growth in the unicellular organism results in increase of cell numbers and so by assessing the cell numbers, the growth of microorganisms can be calculated. Reproduction in bacteria mostly occurs by binary fission where each mother cell divides to form two daughter cells. The time taken by a population of bacterial cells to double its size (numbers) is called doubling time or Generation time. This is the unit of measurement of growth rate. Growth rate, therefore is the time taken by all the cells to reproduce depending on several physiology factors.

Materials requirement:

24 hours broth culture of given bacteria sample.

Sterile liquid & solid nutrient media.

Petri plate, culture tubes, inoculation loop, spirit Lamp, incubator, spectrophometer

Procedure:

1. Take 10 culture tubes and fill them with 5ml of Nutrient broth. Number them accordingly.
2. Keep one tube as blank (only medium without inoculum)**.**
3. Switch on the spectrophotometer before starting the experiment. Set 100% transmission by using the blank.
4. Inoculate the tubes with one ml of inoculum and immediately measure the optical density of first tube at 600m.
5. Incubate the tubes at 37'c.
6. Measure the optical density of second tube after one hour, third tube after two hours and so on.
7. Plot a graph between optical density and time. Time gives the growth curve.

Note:

To find the numbers of cells, compare the obtained optical density with reference curve drawn between optical density and no of the cells.

Study of effect of environment (temperature) on bacteria growth

Aim:

To determine the effect of temperature on the growth of given bacterial culture.

Principle:

Although bacteria are ubiquitous, specific species have specific optional environmental requirements. Any change in such environment requirement impacts the bacterial growth in both positive and negative ways. Among the environmental factors affecting bacterial growth, temperature influence bacterial growth physically. If governs growth, maturation, metabolism, survivability of bacterial cells. On the basis of preferred temperature, bacteria can be classified as follows.

Psychrophiles or cold loving- these bacteria grow between 15'c and 20'c. Some can grow at 0'c.

Mesophiles- these bacteria grow best between 25'c and 40'c. They form a major part of commensals in human body.

Thermophiles or heat loving – these bacteria grow best between 50'c and 60'c and are usually found in boiling hot springs.

Materials requirement:

24 hours bacterial culture (*E. coli*, *Bacillus* sp. or *Pseudomonas* sp.), glucose broth culture tubes, inoculation loop and incubator.

Procedure:

1. Fill the culture tubes with glucose broth.
2. Prepare a set off five tubes for the given bacterial sample and properly label them with the name of the organism and temperature of incubation.
3. Inoculate the tubes with a loop full of respective microbe organisms.
4. Incubate each of the labeled tubes at 5 °C, 20 °C, 37 °C, 45 °C and 55 °C for 24 hours to 48 hours.
5. Observe for bacterial growth and tabulate the results.

Note:

Based on the bacterial growth in a medium expressed the cells as psychrophiles, mesophiles or thermophiles.

Effects of environment factors (pH) on the bacterial growth

Aim:

To determine the influence of pH of the growth medium on the growth of the given bacterial sample.

Principle:

pH is defined as the negative logarithm of the Hydrogen ion concentration of specific solution. It is essential for maintaining the physiological activities of a microorganism. The pH of the growth medium significantly influences the microbial growth under in vitro conditions. On the basis of medium's pH bacteria can be classified as follows.

- Acidophiles or acid loving- these bacteria grow best at ph of 1.0 to 5.4.
  Ex. Lactobacillus
- Neutrophile- these bacteria grow at an optimum pH ranging from 5.4 to 8.5.
  Ex. E. coli
- Alkaliphiles or base loving- these bacteria grow optimally at pH ranging from 7 to 11.5.
  Ex. Vibrio cholera

Material required:

24 hours bacterial cultures (E. coli or Bacillus sp. or Pseudomonas sp. or streptococcus sp.), nutrient agar plates, culture tubes, inoculation, 1N NaOH, 1N Hcl, incubator.

Procedure:

1. Prepare nutrient agar medium in four separate conical flasks.
2. Adjust the pH of the medium as 3, 5, 7 and 9 by using 1N NaOH and/or 1N Hcl.
3. After pH adjustment, sterilize media and pour into respective labeled plates.
4. Maintain an agar plate for each pH as control.
5. Inoculate the given microbial culture using Quadrant streak method and incubate the plate at 37 °C for 24-48 hours.

Note:

Inoculate the media with a high suspension of bacteria in order to prevent false positive and overcrowding effect.

Effects of carbon source on the growth of *E. coli*

Aim:

To determine the effect of carbon source in the culture media on the growth of *E. coli*.

Principle:

Nutrition in the culture media plays an important role in the growth of micro organism. Among the nutrient requirement Carbon Source is an important factor deciding the growth of microbes. Carbon serves as energy sources of micro organisms and help in building up of new cells. Deficiency of shortage of Carbon contents in the nutrient media limits the microbial growth, hence a defined quantity of carbon should be incorporated in the nutrient media in order to facilitate optimal microbial growth. Based on the requirement of carbon as nutrient, the microorganisms are classified as follows.

- Autotrophs- obtain Carbon from CO_2
- Heterotrophs- obtain Carbon from organic compounds
- Chemotrophs- obtain carbon from oxidation of inorganic compounds.
- Photoautotrophs- uses solar energy and nutrients in the medium for carbon utilization.

Material required:

24 hours broth culture of *E. coli***,** incubator**,** inoculation loop**,** Nutrient broth media, glucose, fructose, maltose, xyllulose, ribose sugars. Phenol red - Indicator.

Procedure:

1. Aseptically prepare nutrient broth media and incorporate 1% of sugar separately.
2. Inoculate a loop full of E. coli into respectively labeled tubes.
3. Incubate the tubes at 37 ◦C for 24 hours.
4. After incubation, the observation of growth was done visually or aid spectrophotometer.

Note:

Add 0.01% of phenol red indicator to aseptically prepared nutrient broth medium.

The change of medium's colour from red to yellow after incubation denotes utilization of sugar as carbon source and acid production.

Effects of nitrogen source on the growth of *E. coli*

Aim:

To determine the effects of nitrogen sources on the growth of *E. coli.*

Principle:

Nitrogen is obtained from nature by the reduction of molecular nitrogen present in the environment. In order to utilize the elemental nitrogen, microbes

Use enzymes on organic and inorganic substances in such a way that they are broken down to provide nitrogen. Nitrogen is involved in biosynthesis of amino acid and also the synthesis of nitrogen bases. Thereby forming on integral part of microbial growth in the environment. The optimal level of nitrogen in the growth medium is essential for the adaptability, maturation and growth of microbial cells.

Material required:

24 hours broth culture of *E. coli*, inoculation loop, peptone yeast extract and glucose, incubator

Procedure:

1. Aseptically prepare nutrient media by individually incorporating 1% of tryptone, Yeast extract and Glucose.
2. Inoculate the given bacteria culture onto the respective tubes.
3. Incubate the tubes at 37 °C for 24 hours.
4. After incubation, observe the tubes for visible growth.

Effects of salt on the growth of *E. coli*

Aim:

To determine the effect of salt concentration on the growth of *E. coli.*

Principle:

Bacteria need an optimal concentration of salts to maintain the ionic balance of the cell. Any change in the salt concentration affects the regularly mechanism of the microbial cell which is reflected by the absence of growth in the nutrient media. Microbes utilize salts by a concentration of salt in the nutrient media leads to imbalance in the cellular metabolism which is termed as Osmotic shock. This condition is characterized by membrane blebbing and cell leakage leading to lysis. Some bacteria resist higher concentration of salts with the help of specialized transporter proteins present in the plasma membrane. Based on the growth characteristics of microbes in the presence of salt, microbes are classified as follows:

1. Halophiles or salt lovers- these microbes inhabit marine and require high concentration or sodium chloride for growth.
2. Osmotolerant- these microbes can grow at high solute concentration.
3. Osmophile- these microbes require high solute concentration for metabolism.

Material required:

24 hours broth culture of *E. coli* (nutrient broth), culture tubes, incubator, inoculation loop, sodium chloride anhydrous

Procedure:

1. Aseptically prepare nutrient broth in given test tube.
2. Incorporate various concentration of sodium chloride (0.5, 5, 15, 25%) of sodium chloride into nutrient broth.
3. Inoculate the given bacterial culture into the respectively lateral tubes and incubate the same at 37 °C for 18 hours.
4. Observe the growth of the bacterium in the respective tubes after incubation and record the results.

Demonstration of alcoholic fermentation

Aim:

To determine the ability of the given microorganism to ferment the substrate into alcohol.

Principle:

Fermentation is a process in which sugar molecules are catabolized by micro organism to produce either acid or alcohol and gas. Based on the end product of fermentation process, it is categorized into two types:

1. Acid fermentation- production of weak acid from the given substance (Lactic acid bacteria produce Lactic acid by Lactose fermentation).
2. Alcohol fermentation- production of alcohol as end product from substrate catabolism (Bacillus sp., Yeast produce alcohol form sugars such as starch, fructose and xylose etc).

Fruit juices serve as source for fermentation as it contains high concentration of sugars. Microorganism such as yeast utilizes these sugars and produces alcohol under aerobic fermentation.

Material required:

Grape fruit, 24 hours of yeast culture, incubator, inoculation loop, Erlen-Meyer flask, glass stopper, cotton.

Procedure:

1. The fruit sample is washed under running top water to remove adherent dirt and soil.
2. Fruit sample are randomly smashed and blended into a semi-solid mixture in a conical flask.
3. 0.1ml of *Saccharomyces cerevisia* is inoculated into the mixture. The mixture is incubated at 30 °C-35 °C (Time of incubation may vary based on alcohol production).
4. Check for the presence or absence of alcoholic aroma in the mixture after incubation

Note:

The amount of alcohol produced by fermentation process may be estimated using rapid alcohol estimation methods.

Demonstration of thermal death time and decimal reduction time *E. coli*

Aim:

To demonstrate the effect of lethal temperature on growth of *E. coli.*

Principle:

Microorganism requires temperature for normal growth and metabolism. Temperature is physical factors which influence the regulatory mechanism of membrane and other structural proteins. Thermal Death time is the measurement of time at which the provided temperature is lethal for microbial growth. By calculating Thermal Death Time, the effect of maximum temperature beyond which the microbial growth is inhabited can be calculated.

Material required:

24 hours Nutrient broth culture of E. coli, culture tubes**,** incubator, inoculation loop, water bath**,** nutrient broth

Procedure:

1. Aseptically prepare nutrient broth in the given culture tubes.
2. Prepare 3 sets of 5 tubes and label them with name of the organism and lethal temperature used. (20, 30, 40, 50, 60) °C.
3. Inoculate the given microbial culture onto the appropriately labeled tubes and heat the tubes for the above given temperature for 10 minutes in a water bath.
4. After heating, immediate cool the tubes by immersing in cold water and incubate the same at 37'C for 24 hours.
5. Observe for growth after incubation.

Note:

Maintain a set of tubes without heat treatment as control.