Experiment Details

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| Department Name | **Biotechnology Engineering** |
| Class | TY |
| Semester | V |
| Subject Name | Fermentation Technology |
| Experiment No. | 1 |
| Experiment Name | **DETERMINATION OF CELL MASS BY DIFFERENT METHODS** |

Version History

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| Sr. No. | Version Number | Created By | Approved By | Date |
| 1 | 1.0 | Shivendra Sharad Patil | Asst. Prof. Rutuparna Karkare | 12/10/2020 |
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AIM:

To study the growth of micro-organism by cell mass and optical density measurements.

THEORY:

Growth can be defined in two ways either it is increase in cell number due to division of cells or increase in cell constituents. Microbial growth refers to the growth of total cell population due to cell divisions. The different methods for determination of biomass can be classified into 3 main categories –

1. General methods – Cell mass determination methods including dry weight, wet weight, Packed Cell Volume, Absorbance (optical density)

Cell number determination methods including direct counting on Petroff-Hausser slide and Haemocytometer, Coulter counter, Most Probable Number (MPN)

1. Method correlating increase in specific component like protein or nucleic acids with the growth
2. Methods correlating growth with metabolic activity like substrate consumption rate or product formation rate

PRE TEST:

Q.1) The doubling phase of the microorganisms in the growth curve is \_\_\_\_\_\_\_\_\_\_ phase.

A. Lag phase B. Exponential phase C. Stationary phase D. Death phase

Q. 2) Which of the following method is preferred for Actinomycetes biomass monitoring?

A. Dry weight B. Optical density C. Coulter counter D. Packed cell volume

Q. 3) The method used for direct cell counting is \_\_\_\_\_\_\_\_\_\_\_\_

A. Dry weight B. Optical density C. Packed cell volume D. Haemocytometer

Q. 4) The statistical technique for cell number determination is \_\_\_\_\_\_\_\_\_\_\_\_\_

A. Most probable number B. Optical density C. Coulter counter D. Packed cell volume

Q. 5) The range of wavelength used for absorbance based method of biomass determination is \_\_\_\_\_\_\_\_\_\_

A. 200 nm to 400 nm B. 600 nm to 660 nm C. 260 nm to 280 nm D. 800 nm to 850 nm

PROCEDURE:

1. Prepare and sterilize 100 ml nutrient broth (given medium) in side arm flask.

2. Inoculate the broth with 10 % v/v of seed medium of given micro-organism.

3. Read the absorbance at 600 nm as initial optical density.

4. Sample out 5 ml of broth in centrifuge tube & keep it in refrigerator.

5. Incubate the culture at 370C in shaker incubator.

6. Read the absorbance after every hour and sample out 5 ml of broth after every 3 hrs. (The reason is the significant measurable increase is observed in absorbance over small time period which is not possible for dry weight.) Plot a correlation curve of absorbance versus dry weight.

7. Centrifuge all tubes at 5000 rpm for 15 min or filter the broth using Whatman filter paper

No.1. Weigh empty dried (for removal of moisture) centrifuge tube or filter paper.

8. Discard the supernatant & centrifuge again by adding 10ml D/W to each tube.

9. Keep the tubes or filter paper in hot air oven (or vacuum oven) at 800C for 24 hrs or 1100C for 5 hrs to obtain dry cell mass.

10. Weigh centrifuge tube or filter paper and calculate the dry cell mass.

11. Plot the changes in both parameters with respect to time.

12. Measure the growth limiting substrate (in most cases Carbon source i.e. total carbohydrate like glucose) initially (at 0 hrs) and finally (after 48 hrs) and calculate the yield coefficient.

POST TEST:

Q.1) The optical method of growth monitoring is based on \_\_\_\_\_\_\_\_\_\_ phenomenon.

A. Molecular absorption B. Molecular emission C. Light Scattering D. Light diffraction

Q. 2) The equipment used in the drying of the biomass in dry weight method is \_\_\_\_\_\_\_\_\_\_\_

A. Microwave oven B. Hot air oven C. Refrigerator D. Vacuum evaporator

Q. 3) Whatman filter paper used for filtration of cells is of grade \_\_\_\_\_\_\_\_\_\_\_\_

A. No. 2 B. No. 5 C. No. 4 D. No. 1

Q. 4) Which of the following biomass estimation method cannot be used directly to calculate the yield coefficient?

A. Wet weight B. Dry weight C. Optical density D. Packed cell volume

Q. 5) The instrument used direct cell number counting is \_\_\_\_\_\_\_\_\_\_\_\_

A. Coulter counter B. Hot air oven C. Spectrophotometer D. Vacuum evaporator

REFERENCES:

**1. Bioprocess Engineering – Basic Concepts by Shuler and Kargi – Pearson Education**

**2. Techniques used in Bioproduct Analysis – Biotol Series (Heinmann-Butterworth Pub.)**

**3. Principles of Fermentation Technology by Stanbury, Whitaker and Hall (Aditya Books Ltd.**)