BIOREACTOR ENGINEERING
Chapter 9
Sterilization in Fermentation

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Chapter Description

- Topic Outcomes
  - Describe appropriate sterilization technique for medium, air and fermenter.
  - Perform sterilization calculation.

- References
  - Doran, P.M. (2013) Bioprocess Engineering Principles. Elsevier.
  - Liu, S. (2013) Bioprocess Engineering: Kinetics, Biosystem, Sustainability and Reactor Design. Elsevier.
  - Rao, D.G. (2010) Introduction to Biochemical Engineering. McGraw Hill.

Topic Outline

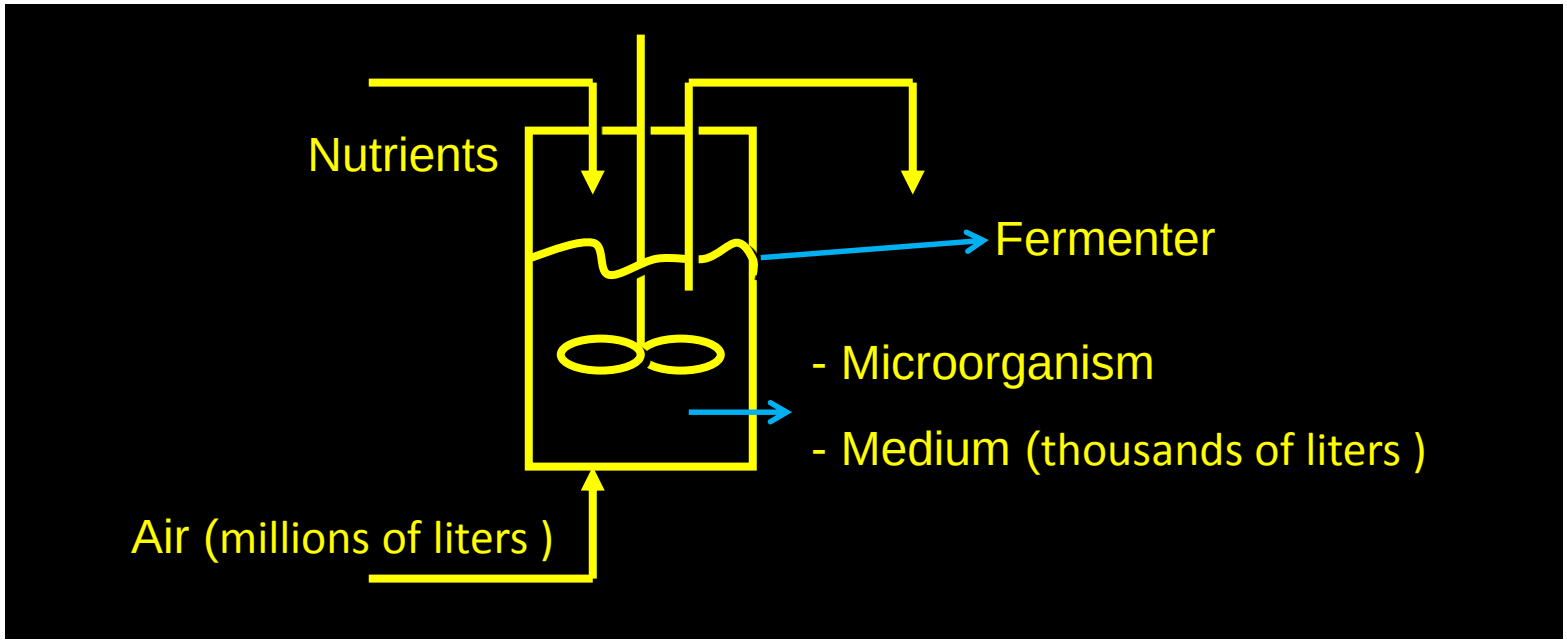
- Introduction
- Medium Sterilization
- Air Sterilization
- Fermenter Sterilization

Introduction

- Effect of contamination on fermentation:
  - Medium is consumed unnecessarily → may affect the growth of desired organism & outweigh the desired product.
  - Fermentation condition is affected → may affect the growth of desired organism & outweigh the desired product.
  - Desired product is contaminated → interfere the downstream process.

Introduction

- Fermentation involves:



- A sterile environment is needed for all the above.

Introduction

- Methods to avoid contamination in a fermentation process:
  - Sterilization of the medium.
  - Sterilization of the fermenter.
  - Sterilization of all materials to be added to the fermenter.
  - Sterilization of air.
  - Employing pure inoculum.
  - Sterilizing the pipes, valves, and bends. which come in contact with the fermentation process.
  - Maintaining aseptic conditions in the fermenters during fermentation.
  - Maintaining optimum or desired pH.

Introduction

- Methods available for sterilization:
  - Chemical - preferred for heat-sensitive equipment
    - ethylene oxide (gas)
    - 70% ethanol
    - 3% sodium hypochlorite
  - Exposure to radiation
    - UV for surface
    - X-ray for liquid
  - Filtration
  - Heating

Medium Sterilization

- Heating method: The medium is heated to the sterilization temperature (121°C).
  - Thermal death kinetics of microorganisms: $\frac{dN}{dt} = -k_d N$

    where,
    N = number of live cell
    t = sterilization time
    k_d = specific death constant
  - For constant k_d, integration: $ln\frac{N_t}{N_o} = -k_d t$

    where,
    N_o = initial number of live cell
    N_t = number of live cell still present after a time period of t

Medium Sterilization

- Exercise 1

Medium Sterilization

- k_d is function of temperature:

$$k_d = Ae^{-\frac{E_d}{RT}}$$

where,
A = Arrhenius constant
E_d = activation energy for thermal cell death
R = ideal gas constant
T = absolute temperature

  - For *Bacillus stearothermophilus*, E_d is about 70 kcal/gmol.
  - For *E. coli*, E_d is about 127 kcal/gmol.
  - For vitamin in medium, E_d is about 2-20 kcal/gmol.
- $E_A > E_B$
  - Increase in temperature will destroy more A compared to B.

Medium Sterilization

- Exercise 2

Medium Sterilization

- Filter method: Suitable for medium containing heat-sensitive components.
  - Membrane: made of cellulose esters or other polymers, pore diameter between 0.2-0.45 µm.
  - Membrane itself must be sterilized by steam or radiation before use.
  - Bacteria and other particles with dimensions greater than the pore size are screened out and collected on the surface of the membrane.
  - Filtration is not as effective or reliable as heat sterilization.

Air Sterilization

- The number of microbial cells in air = $10^3 – 10^4$ /m^3
- Method of air sterilization:
  - Sterilization by heating (economically impractical).
  - Radiation (UV rays).
  - Use of germicidal sprays (e.g., phenol, ethylene oxide or formalin).
  - Sterilization by filtration.
- Filtration sterilization is commonly used.

Air Sterilization

- Filter is also used to sterilize off-gases leaving the fermenter.
  - The concentration of cells in unfiltered fermenter off-gas is several times greater than in air.
  - Organisms (e.g., pathogens) are harmful to plant personnel or environment.
- Two types of air filter:
  - Depth filter (Fibrous filter): Filter with pores that are bigger than the size of the microorganism to be removed.
  - Surface filter (Absolute filter): Filter with pores that are smaller than the size of the microorganism to be removed.

Fermenter Sterilization

- Any joints, crevices, pits or flange joint are potential hazardous points where the nutrients and microorganism stay, hence contamination can take place.
- Fermenter should be free of crevices and stagnant areas, have minimum number of joints, polished welded joints are used, joint point should be as smooth as possible.
- Industrial fermenters are designed for in situ steam sterilization under pressure (15 psig for 20 min).
- After sterilization, the fermenter should be flushed with sterile air to keep under positive pressure.
- After sterilization, sterile medium and air entering fermenter must be sterile. The transport line should be maintained under aseptic conditions.

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