Fermentor

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What is fermentation?

- Pasteur’s definition: “life without air”, anaerobe red ox reactions in organisms
- New definition: a form of metabolism in which the end products could be further oxidized

For example: a yeast cell obtains 2 molecules of ATP per molecule of glucose when it ferments it to ethanol
What is fermentation technique?

Techniques for large-scale production of microbial products. It must both provide an optimum environment for the microbial synthesis of the desired product and be economically feasible on a large scale. They can be divided into surface (emersion) and submersion techniques. The latter may be run in batch, fed batch, continuous reactors.

In the surface techniques, the microorganisms are cultivated on the surface of a liquid or solid substrate. These techniques are very complicated and rarely used in industry.
What is fermentation technique?

In the submersion processes, the microorganisms grow in a liquid medium. Except in traditional beer and wine fermentation, the medium is held in fermenters and stirred to obtain a homogeneous distribution of cells and medium. Most processes are aerobic, and for these the medium must be vigorously aerated. All important industrial processes (production of biomass and protein, antibiotics, enzymes and sewage treatment) are carried out by submersion processes.
Typical Bioprocessing

Stock Culture

Shake Flasks

Seed Fermenter

Raw Materials

Medium Formulation

Sterilization

Air

Agitator

Recovery

Purification

Products
View looking down into a stainless steel fermentor
AGITATOR (IMPELLER)

Achieve **mixing objectives** — bulk fluid and gas-phase mixing, air dispersion, oxygen transfer, heat transfer, suspension of solid particles and maintaining uniform environment throughout vessel contents.
Introduction

The function of the fermenter or bioreactor is to provide a suitable environment in which an organism can efficiently produce a target product—the target product might be:

- Cell biomass
- Metabolite
- Bioconversion Product

The sizes of the bioreactor can vary over several orders of magnitudes.

The microbial cell culture (few mm$^3$), shake flask (100 - 1000 ml), laboratory fermenter (1 - 50 L), pilot scale (0.3 - 10 m$^3$) to plant scale (2 - 500 m$^3$) are all examples of bioreactors.
Cell culture fermenter

Yeast Fermentation

- grape juice + yeast (wine)
- glucose $\rightarrow$ ethyl alcohol + \( \text{CO}_2 \) + ATP

Shake flask fermenter

Pilot fermenter

laboratory fermenter

Plant fermenter
Upstream Processing

- Three main areas:
  - A) Producer microorganism
  - This include processes for
    - obtaining a suitable microorganism
    - strain improvement to increase the productivity and yield
    - maintenance of strain purity
    - preparation of suitable inoculum
  - B) Fermentation media
  - C) Fermentation Process
Downstream Processing

- The processes that follow fermentation:
  - A) Cell harvesting
  - B) Cell disruption
  - C) Product purification from cell extracts or the growth medium
Key Factor of Fermenter design

- The performance of any fermenter depends on the following key factors:
  - Agitation rate
  - Oxygen transfer
  - pH
  - Temperature
  - Foam production
- The design and mode of operation of a fermenter mainly depends on the production organism, the optimal operating condition required for target product formation, product value and scale of production.
- The design also takes into consideration the capital investment and running cost.
Requirements of Bioreactors

- There is no universal bioreactor.
- The general requirements of the bioreactor are as follows:
  - A) The design and construction of bioreactors must keep sterility from the start point to end of the process.
  - B) Optimal mixing with low, uniform shear.
  - C) Adequate mass transfer, oxygen.
  - D) Clearly defined flow conditions.
  - E) Feeding substrate with prevention of under or overdosing.
  - F) Suspension of solids.
  - G) Gentle heat transfer.
  - H) Compliance with design requirements such as: ability to be sterilized; simple construction; simple measuring, control, regulating techniques; scale-up; flexibility; long term stability; compatibility with up- downstream processes; antifoaming measures.
Some important fermentation products

<table>
<thead>
<tr>
<th>Product</th>
<th>Organism</th>
<th>Use</th>
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<tbody>
<tr>
<td>Ethanol</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Industrial solvents, beverages</td>
</tr>
<tr>
<td>Glycerol</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Production of explosives</td>
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<tr>
<td>Lactic acid</td>
<td><em>Lactobacillus bulgaricus</em></td>
<td>Food and pharmaceutical</td>
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<tr>
<td>Acetone and butanol</td>
<td><em>Clostridium acetobutylicum</em></td>
<td>Solvents</td>
</tr>
<tr>
<td>α-amylase</td>
<td><em>Bacillus subtilis</em></td>
<td>Starch hydrolysis</td>
</tr>
</tbody>
</table>
Some important fermentation products

leucine

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\begin{align*}
&\text{H}_3\text{C} - \text{C} - \text{CH}_3 \\
&\text{CH}_2 \\
&\text{H}_2\text{N} - \text{C} - \text{COOH} \\
&\text{H}
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glutamic acid

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\begin{align*}
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&\text{C} \\
&\text{CH}_2 \\
&\text{CH}_2 \\
&\text{H}_2\text{N} - \text{C} - \text{COOH} \\
&\text{H}
\end{align*}
\]

lysine

\[
\begin{align*}
&\text{NH}_2 \\
&\text{CH}_2 \\
&\text{CH}_2 \\
&\text{CH}_2 \\
&\text{H}_2\text{N} - \text{C} - \text{COOH} \\
&\text{H}
\end{align*}
\]
Some important fermentation products
Some important fermentation products
Winemaking fermenter
Flow sheet of a multipurpose fermenter and its auxiliary equipment
Component parts of a fermentation

1. **Formulation of media** to be used in culturing the organism during development of inoculum and in the production fermenter

2. **Sterilization** of the medium, fermenter and ancillary equipment

3. **Production of** an active, **pure culture** in sufficient quantity to inoculate the production vessel

4. The **growth of the organism in** the production **fermenter** under optimum conditions for product formation

5. The **extraction of the product** and its purification

6. **Disposal of effluents** produced by the process
Typical fermentation process involves:

- **Upstream processing (USP)**
- **Downstream processing (DSP)**

USP is associated with all factors and processes leading to and including the fermentation.

Consists of three main areas:

1. The producer microorganism
2. The fermentation medium
3. The fermentation
DSP includes all processes that follow fermentation. They involve:

1. cell harvesting
2. cell disruption
3. product purification from cell extracts or the growth medium
4. Disposal of effluent wastes
APPLICATIONS OF FERMENTATION

Microbial fermentations may be classified into the following major groups:-

- (i) Those that produce microbial cells (biomass) as the product.
- (ii) Those that produce microbial metabolites.
- (iii) Those that produce microbial enzymes.
- (iv) Those that modify a compound which is added to the fermentation - the transformation processes.
- (v) Those that produce recombinant products.
Microbial Growth Kinetics

- Microbial Growth Kinetics describe how the microbe grows in the fermenter. This information is important to determine optimal batch times. The growth of microbes in a fermenter can be broken down into four stages:
  - Lag Phase
  - Exponential Phase
  - Stationary Phase
  - Death Phase
Microbial Growth Kinetics

• Lag Phase
  • This is the first phase in the fermentation process
  • The cells have just been injected into a new environment and they need time to adjust accordingly
  • Cell growth is minimal in this phase.
Microbial Growth Kinetics

- Exponential Phase
  - The second phase in the fermentation process
  - The cells have adjusted to their environment and rapid growth takes place
  - Cell growth rate is highest in this phase
Microbial Growth Kinetics

• Exponential Phase (Continued)
  • At some point the cell growth rate will level off and become constant
  • The most likely cause of this leveling off is substrate limited inhibition
    • Substrate limited inhibition means that the microbes do not have enough nutrients in the medium to continue multiplying.
Microbial Growth Kinetics

• Stationary phase
  • This is the third phase in the fermentation process
  • The cell growth rate has leveled off and become constant
  • The number of cells multiplying equals the number of cells dying
Microbial Growth Kinetics

• Death phase
  • The fourth phase in the fermentation process
  • The number of cells dying is greater than the number of cells multiplying
    • The cause of the death phase is usually that the cells have consumed most of the nutrients in the medium and there is not enough left for sustainability
• Most fermentations require liquid media, often referred to as broth

• Fermentation media must satisfy all the nutritional requirements of the microorganism

• Most fermentations, except those involving solid substrates, require large quantities of water in which the medium is formulated.
General media requirements include

- a carbon source - provides both energy and carbon units for biosynthesis
- sources of nitrogen, phosphorus and sulphur.
- Other minor and trace elements
- some microorganisms - vitamins, such as biotin and riboflavin.

- Aerobic fermentations are dependent on a continuous input of molecular oxygen, and even some anaerobic fermentations require initial aeration of media, e.g. beer fermentations
• buffers, or the pH is controlled by acid and alkali additions

• antifoam agents

• For some processes, precursor, inducer or inhibitor compounds must be introduced at certain stages of the fermentation.
The main factors that affect the final choice of individual raw materials are as follows.

1 Cost and availability
2 Ease of handling in solid or liquid forms, along with associated transport and storage costs
3 Sterilization requirements and any potential denaturation problems.
4 Formulation, mixing, and viscosity - characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages.
5 The concentration of target product attained, its rate of formation and yield of product per gram of substrate utilized.
6 The levels and range of impurities, and the potential for generating further undesired products during the process.
7 Overall health and safety implications.
Control of Physicochemical Parameters

- **A) Agitation:**
  - Agitation of suspended cell fermentations is performed in order to mix the three phases within a fermenter.
  - **liquid phase** contains dissolved nutrients and metabolites.
  - gaseous phase is predominantly oxygen and carbon dioxide.
  - **solid phase** is made up of the cells and any solid substrates that may be present.
  - Mixing should produce homogeneous conditions and promote:
    - a) Nutrient transfer
    - b) Gas transfer
    - c) Heat transfer
  - Heat transfer is necessary during both sterilization and for temperature maintenance during operation.
AGITATION

• Helps to mix 3 phases within a fermentor
  liquid phase-nutrients and metabolites
  gaseous phase-O2 and CO2
  solid phase-cells any solid substrates present

• Mixing – homogenous conditions and promote nutrient, gas and heat transfer

• Heat transfer- sterilization and temp maintenance during fermentation process
• Aerobic fermentations- mixing for transfer of O2 from gaseous to liquid phase.
• Stirred tank reactors-agitators or impellors are used for agitation
• Impellor-connected to a strong and straight shaft
• Shaft passes through the lid of the fermentation tank
• Shaft rotated with electric motor mounted externally on top of the fermentor
• Appropriate seals
Effectiveness of agitation depends on design of impeller blades, speed of agitation, and depth of liquid.

Vortex formation—reduced agitation and reduced aeration as only a small amount of the medium surface is exposed to the atmosphere in the head space of the tank.

To avoid this problem, flat vertical plates—width of 1/10th of the vessel called baffles.

Baffles are present on the wall of the fermentor.
Disc type
Marine type
Inclined type
Open turbine
• They increase turbulence, prevent vortex formation and eliminates dead spaces.
• Usually 4-6 baffles per fermentor
• Agitation should be controlled to suite a particular fermentation
• High shear- damage shear sensitive cells
• Low shear agitation systems – flocculation of cells or unwanted growth on the surfaces such as vessel walls, stirrers and electrodes
• Disadvantages of mechanical agitation systems:
  - Damage to shear sensitive cells
  - High initial costs
  - Maintenance costs
  - Higher power consumption
  - Excessive foam formation

• Advantages of mechanical agitation systems:
  - Efficient mixing of contents
  - Proper distribution of dissolved O2, temp, and pH
• Other systems of agitation - Airlift fermentor
• No moving parts
• Uses expansion of compressed gas to bring about the mixing
• Liquid movement initiated by injection of compressed air at the bottom of the column
• Air bubbles expand in column causing the upward movement of liquid – initiate cycling within the fermentor.
Achieve mixing objectives – bulk fluid and gas-phase mixing, air dispersion, oxygen transfer, heat transfer, suspension of solid particles and maintaining uniform environment throughout vessel contents.
**Baffles**

- Four baffles incorporated into agitated vessels of all sizes to **prevent vortex** and to **improve aeration efficiency**
- Metal strips roughly one-tenth of vessel diameter and attached radially to the wall
- Minimizes microbial growth on baffles and fermenter walls.
Control of Physicochemical Parameters

B. Transport of Oxygen

To prevent the risk of contamination, gases introduced into the fermenter should be passed through a sterile filter.

A similar filter on the air exhaust system avoids environmental contamination.

Sterile filtered air or oxygen normally enters the fermenter through a sparger system,

To promote aeration in stirred tanks, the sparger is usually located directly below the agitator.
AERATION SYSTEM (SPARGER)

Introduces **air into liquid of fermenter**

Three basic types – **porous sparger**

1. **Orifice sparger** – a perforated pipe
2. **Nozzle sparger** – an open or partially closed pipe
3. **Combined sparger-agitator**
Aeration

- Majority of fermentations are aerobic
- Requires large quantities of sterile air or O2
- Also removes unwanted volatile metabolic products from the medium
- Air or gases - passed through filter???
- Filter at the exhaust ???
• Sterile air enters the fermentor through a sparger system
• Air flow rarely exceeds 0.5 to 1.0 volume of air per volume of medium
• Stirred tank-sparger is placed directly below the agitator
• Different kinds of sparger used
• Smaller the size of air bubbles produced more efficient is the aeration
Oxygen supply

Oxygen is normally supplied to microbial cultures in the form of air, this being the cheapest available source of the gas. The method for provision of a culture with a supply of air varies with the scale of the process:

i. Laboratory-scale cultures may be aerated by means of the shake-flask technique where the culture (50 to 100 cm$^3$) is grown in a conical flask (250 to 500 cm$^3$) shaken on a platform contained in a controlled environment chamber.

ii. Pilot and industrial-scale fermentations are normally carried out in stirred, aerated vessels, termed fermenters.
Ring type
Grid type
• Ring sparger and grid sparger—used when unicellular organisms are used for fermentation
• Multicellular organisms clog the holes
• Single long pipe used to pump air to avoid clogging
• Or ring sparger with orifice facing downward can also avoid clogging
Antifoams

- Antifoams are necessary to reduce foam formation during fermentation.

- Foaming is largely due to media proteins that become attached to the air–broth interface where they denature to form a stable foam.

- Proteolytic bacteria

- If uncontrolled the foam may
  1) block air filters, resulting in the loss of aseptic conditions; the fermenter becomes contaminated and microorganisms are released into the environment
2) Considerable loss of medium

3) Reduces aeration by hindering gas exchange b/w medium and atmosphere in head space
The ideal antifoam should have the following properties:

- 1 readily and rapidly dispersed with rapid action;
- 2 high activity at low concentrations;
- 3 prolonged action;
- 4 non-toxic to fermentation microorganisms, humans or animals;
- 5 low cost;
- 6 thermostability; and
- 7 compatibility with other media components and the process, i.e. having no effect on oxygen transfer rates or downstream processing operations.
There are three possible approaches to controlling foam production:

- modification of medium composition,
- use of mechanical foam breakers
- addition of chemical antifoams
• Mechanical foam control- impeller blade
  ultrasonic waves
• Chemical antifoams- added manually or can be automated
• Natural antifoams include plant oils (e.g. from soya, sunflower and rapeseed), deodorized fish oil, mineral oils.

• The synthetic antifoams are mostly silicon oils, poly alcohols and alkylated glycols.
Transfer of Heat in Bioreactors

- To maintain a constant temperature in the fermenter, heat is either supplied or removed from the fermentation broth during the course of fermentation.

- In fixed bed microbial reactors heat transfer takes place by natural convection or phase change (evaporation-condensation).
• **Heat Transfer Configurations:**

• The primary heat transfer configurations in fermentation vessels are:
  
  i. External jackets
  
  ii. Internal coils
  
  iii. External surface heat exchanger

• The internal coils though provide better heat transfer capabilities, but they cause problems of microbial film growth on coil surfaces, alteration of mixing patterns and fluid velocities.
VALVES

• To control the flow of liquids and gases
• Valves may be:-
  • Simple on / off-fully open or fully closed
  • Coarse control of flow rates
  • Valves that can be adjusted precisely-flow rates can be controlled accurately
• Safety valves
Gate valves

- Sliding disc is moved in or out of the path by turning the stem of the valve
- Suitable for general purposes on a steam or water line
- Fully open or fully closed
- Should not be used for regulating flow
- Not suitable for aseptic conditions
  ----mash solids can pack in the groove where the gate slides
  ----Leakage round the stem of the valve
Globe valves

- Horizontal disc or plug is raised or lowered in its seating
- Regulates the flow of water or steam
- Not suitable for aseptic operation—leakage round the valve stem(similar to gate valve)
- Flexible metallic membrane around the stem of gate and globe valves—aseptic operations—but expensive
Piston valves

- Similar to globe valve
- Except flow is controlled by a piston passing between two packing rings
- Very efficient for aseptic operation
Needle valves

- Similar to globe valve
- Except-disc is replaced by a tapered plug or needle fitting into a tapered valve seat
- Used to give fine control of steam or liquid flow
- Not suitable for aseptic operations
Plug valves

- Parallel or tapered plug sitting in a housing through which an orifice has been machined
- When plug is turned through 90 degree the valve is fully open
- Flow path determined by the cross-sectional area of the orifice
- Orifice is not as large as that of the pipeline
Ball valves

- Developed from the plug valve
- Valve is a stainless steel ball through which an orifice is machined
- Ball is sealed between two wiping surfaces
- Wipe the surface and prevent deposition of matter at this point
- Orifice – same diameter as that of the pipe line, giving an excellent flow path
- Suitable for aseptic operations
- Can handle mycelial broths
- Can be operated under high temperatures and pressures
Butterfly valves

- Consists of a shaft which rotates about a shaft in a housing
- Disc closes against a seal to stop the flow of liquid
- Valves usually used in large diameter pipes operating under low pressure where absolute closure is not essential
- Not suitable for aseptic operations
- Neck (SCS13A)
- Stem (SUS403 or SUS410, SUS420J2Q for 250A and larger)
- O-ring (EPDM)
- Seat (EPDM)
- Body (ADC12, AC4C for 250A and larger)
- Disc (SCS14A)
Pinch Valves

- Flexible sleeve closed by a pair of pinch bars
- Suitable for aseptic operations
Diaphragm valves

- Flexible closure
- Suitable for aseptic operations
- Diaphragm must be made of a material that can withstand repeated sterilization
Check valves

- Prevent accidental reversal of flow of liquid or gas in a pipe due to breakdown in some part of the eqpt
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<td>1</td>
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<td>2</td>
<td>BOLT</td>
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<td>4</td>
<td>HINGE PIN</td>
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<td>5</td>
<td>SEAT RING</td>
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<td>6</td>
<td>HINGE</td>
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<td>7</td>
<td>DISC</td>
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Pressure control valves

- Pressure reduction valves
- Pressure retaining valves
- Safety valves
Safety valve
Types of fermenter

- Simple fermenters (batch and continuous)
- Fed batch fermenter
- Air-lift or bubble fermenter
- Cyclone column fermenter
- Tower fermenter
- Other more advanced systems, etc

The size is few liters (laboratory use) - >500 m³ (industrial applications)
Tower fermentor

- Elongated non mechanically stirred fermentor
- 10:1 diameter ratio
- Unidirectional flow of gases
- Tube with sparger at the base
- Initially used in the batch fermentor mode-citric acid production
- Later in 1965- continuous mode for brewing industry
• *Settling zone or separator*- at the top of the fermentor- induces the gas bubbles produced during the reaction to coalesce and escape from the liquid phase

• *Quiescent zone* within the separator – free of rising gas – so that yeast cells could settle and return to the main body of the tower and clear beer can be removed
• Attemporator jacket- encloses the tower temperature regulation of the contents
• Little yeast lost due to the flocculant nature of the yeast
• Wort is introduced into the base of the fermentor and it passes through a porous plug of yeast
• Yeast concentration is 30-35% by weight at the bottom of the tower and as low as 5-10% at the top due to the flocculent nature of the yeast
• Progressive and continuous fall in specific gravity of the nutrient medium between the bottom and the top of the tower???

• Steam sterilized b4 fermentation

• Vessel filled partially with sterile wort and inoculated with flocculant yeat

• Initial stages- designed for high biomass production----- by periodic addition of wort for about 9 days

• Porous plug of yeast develops at base of the tower
• Flow rate of the wort is gradually increased over the next 9-12 days and a steady state is achieved in this time.
• Beer produced in the initial 3 weeks does not contain sufficient alcohol concentration.
• Thus more than 3 months operations necessary to compensate for the initial losses of the process.
Air lift fermenter
• Advantage: low energy requirement
  create less shear than stirred tank reactors
• Deep jet fermentor: pump is used externally for circulation and reinjection
• Smaller the bubble, larger the surface area to volume ratio and better O2 transfer to the liquid phase
• Spargers with small pores-prone to blockage, energy requirement is high
• Size of the holes ranges from 1/64 to 1/32 of an inch or larger
• Tall fermentor aeration is better as bubbles remain longer in the medium
그림 9.9. 다실형 원심분리기의 개략도