Chapter 4: Industrial Fermentations

1. Definition and context of fermentation

The term **fermentation** is used in two different contexts by microbiologists:

1.1 Metabolic definition

In metabolism, fermentation refers to an **energy-generating process** in which organic compounds act as both electron donors and electron acceptors. This is an anaerobic process that does not involve an electron transport chain. Examples include lactic acid fermentation and alcoholic fermentation.

1.2 Industrial definition

In industrial microbiology, fermentation refers to the **large-scale growth of microorganisms** (or plant/animal cells) under **aerobic or anaerobic conditions** inside a closed vessel called a **fermenter** or **bioreactor**. The goal may be to produce:

- Cell biomass (e.g., baker's yeast)
- Metabolites (e.g., antibiotics, organic acids)
- Bioconversion products (e.g., transformed steroids)

2. Types of fermentation systems

Fermentations are classified according to several criteria. The choice of system depends on the microorganism, product value, and risk of contamination.

2.1 Based on biological phase organization

Type	Description	Example
Suspended growth	Cells are freely dispersed in liquid medium, as single cells or flocs	Bacterial cultures, yeast
Supported growth	Cells develop as a biofilm on an inert support	Wastewater treatment, some food fermentations

Supported growth is further divided into:

- **Fixed film:** Liquid flows over static support
- **Fluidized/expanded bed:** Support particles are suspended in liquid

2.2 Based on sterility

- **Aseptic (sterile) fermentations:** Used for high-value products (pharmaceuticals, recombinant proteins). All inputs are sterilized.
- **Non-aseptic fermentations:** Used for traditional products (beer, wine, some foods). Relies on extreme pH, high temperature, or protected substrates to prevent contamination.

2.3 Based on aeration

- **Aerobic:** Requires continuous oxygen supply (e.g., antibiotic production)
- **Anaerobic:** No oxygen required (e.g., ethanol production)

Table 6.1 Examples of aseptic and non-aseptic fermentations

Aseptic		Non-aseptic	
Aerobic	Anaerobic	Aerobic	Anaerobic
Animal and plant cell cultures	Acetone	Acetification of ethanol in vinegar production*	Alcoholic beverages; beer, wine, etc.*
Alkaloids	Butanol	Ripening of some cheeses	Primary dairy fermentations*
Amino acids	Ethanol	Mushroom production	Silage production
Most antibiotics	Glycerol	Aerobic waste-water treatment	Anaerobic waste-water treatment
Most biomass (SCP) production	Lactic acid		
Most enzymes	Some toxins		
Most organic acids			
rDNA proteins			
Steroid biotransformations			
Some toxins			
Most vaccines			
Most vitamins			
Xanthan gum			

* Usually a clean operation often referred to as 'commercially sterile'.

3. Fermenter design and construction

A bioreactor (or fermenter) is, by definition, an enclosure made of **glass or stainless steel** allowing optimal growth of microorganisms and optimal production in an environment where the physical and chemical parameters of fermentation are controlled. It includes:

- A culture vessel, made of glass or stainless steel, with a variable volume ranging from a few liters to several cubic meters in industrial units. The tank is hermetically sealed.
- An agitation system (depending on the case) is used to ensure mixing and aeration of the culture; it consists of an external motor and one or more internal turbines (depending on the size of the fermenter).
- A syringe to inject the culture medium or nutrients.
- Probes for monitoring temperature (thermometer), pH (pH meter), dissolved oxygen concentration (oxymetric probe).
- A control unit managed by a computer records and controls all operating parameters.

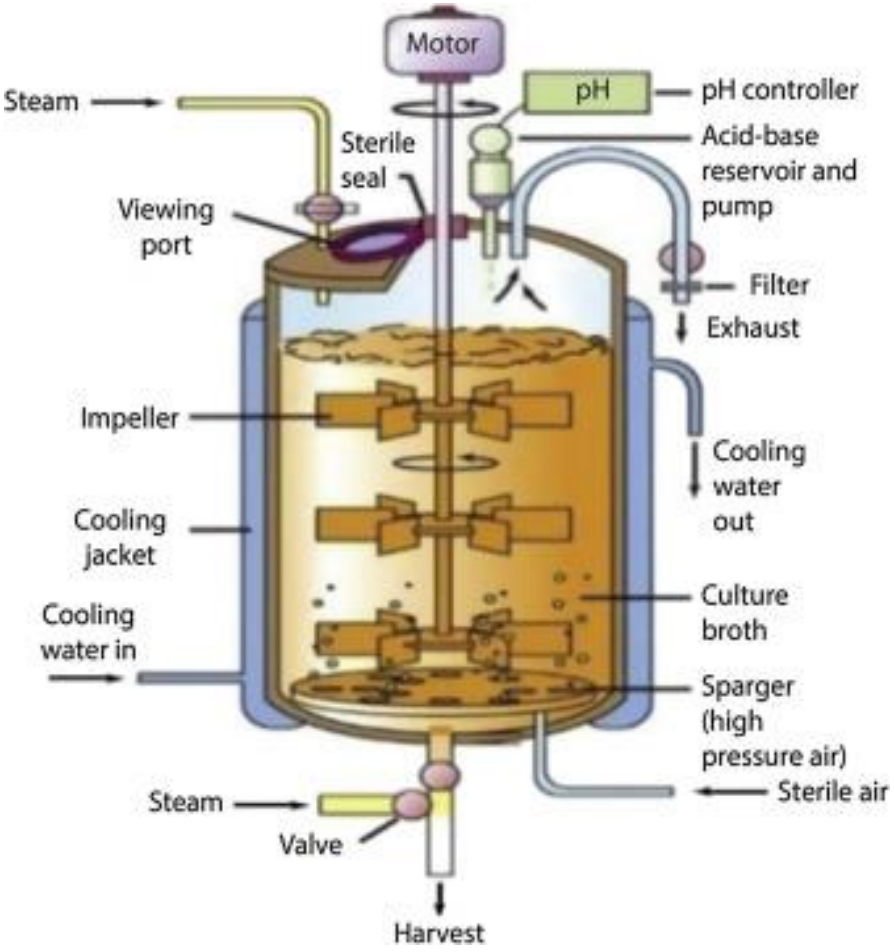


Fig. Schematic of an industrial-scale fermenter (Singhal et al., 2018).

3.1 Main function

Provide a **controlled environment** that allows an organism to efficiently produce a target product.

Note: Bioreactors are classified according to their maximum volume:

- Autoclavable laboratory bioreactors: up to 18 L;
- *In situ* sterilizable laboratory bioreactors: up to 30 L;
- Pilot-scale bioreactors: up to 300 L;
- Industrial-scale bioreactors: up to 500,000 L (500 m^3).

3.2 Key physical and chemical parameters to control

- Agitation rate
- Oxygen transfer
- pH
- Temperature
- Foam production

3.3 Materials of construction

Scale	Material
Laboratory (few liters)	Glass and/or stainless steel
Pilot scale	Stainless steel (polished interior)
Industrial (very large)	Mild steel lined with glass or plastic (to reduce cost)

3.4 Aseptic design requirements

To maintain sterility for long periods:

- **No direct contact** between sterile and non-sterile sections
- All pipelines (air, inoculum, nutrients) must be **steam-sterilizable**
- **Aseptic inoculation, sampling, and harvesting** systems
- **CIP (Cleaning-In-Place):** Automated spray jets inside the vessel
- **No horizontal pipes, unnecessary joints, or dead spaces** (prevents microbial accumulation)
- **Butt-welded joints** with polished inner surfaces (instead of flanged joints)
- **Pressure gauges and safety burst discs** (not spring-loaded valves, which risk contamination)
- **Avoid pumps** if possible; if necessary, use peristaltic, magnetic, or jet pumps
- **Condenser** if volatile compounds are present
- **Filter-sterilized exhaust gases** to contain aerosols

4. Agitation

4.1 Purpose

Mix the **three phases** inside a fermenter:

- **Liquid:** dissolved nutrients and metabolites
- **Gas:** oxygen and carbon dioxide
- **Solid:** cells and solid substrates

Good mixing ensures:

- Homogeneous conditions
- Nutrient, gas, and heat transfer
- Prolonged bubble retention (in aerobic cultures)
- Smaller bubble size → larger surface area for oxygen transfer

4.2 Three principal agitation mechanisms

4.2.1 Stirred tank reactor (STR)

- **Mechanism:** Mechanical impeller inside a baffled cylindrical vessel
- **Baffles:** 4–6 flat vertical plates (width ≈ 1/10 vessel diameter) to prevent vortex and dead spaces
- **Shear:** High

- **Common use:** Most widely used fermenter type
- **Aseptic seals:** Required where the shaft enters the vessel (2–3 seals for high-risk organisms)
- **Aspect ratio:** Typically 3:1 or 4:1 (height:diameter)

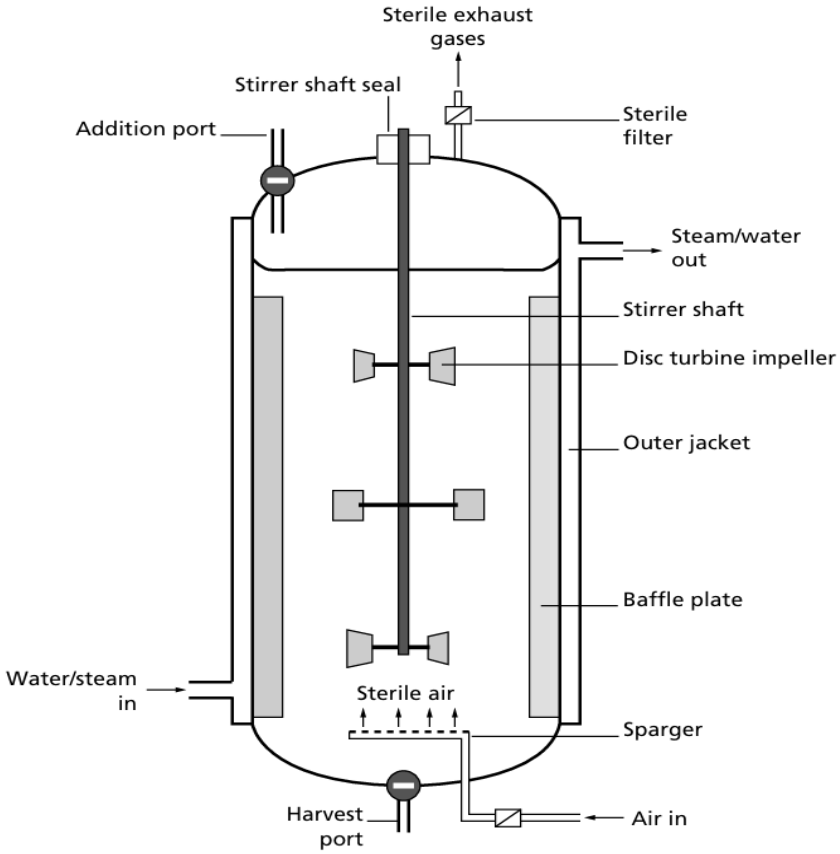


Fig. Diagram of a stirred tank reactor.

4.2.2 Pneumatic systems (airlift fermenter)

- **Mechanism:** Compressed air injected at the bottom of a riser column; bubbles expand and cause liquid circulation
- **Shear:** Low
- **Advantages:** No moving parts, lower energy, less shear damage
- **Cooling:** Jacket usually sufficient (no internal coils needed)

4.2.3 Hydrodynamic systems (deep-jet fermenter)

- **Mechanism:** External liquid pump circulates and reinjects liquid
- **Shear:** Variable
- **Use:** When high mixing energy is required

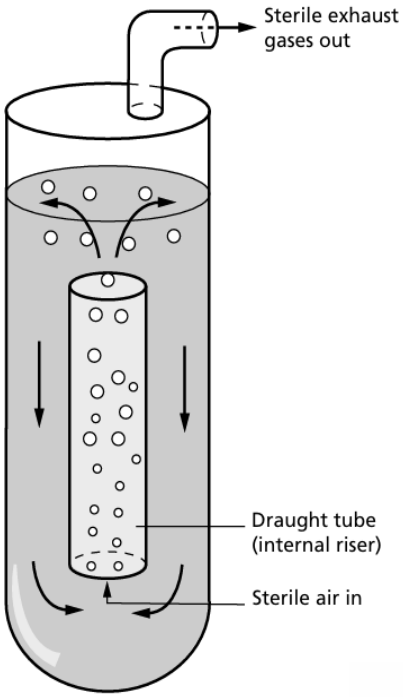


Fig. 6.2 A diagram illustrating the principle of an airlift fermenter.

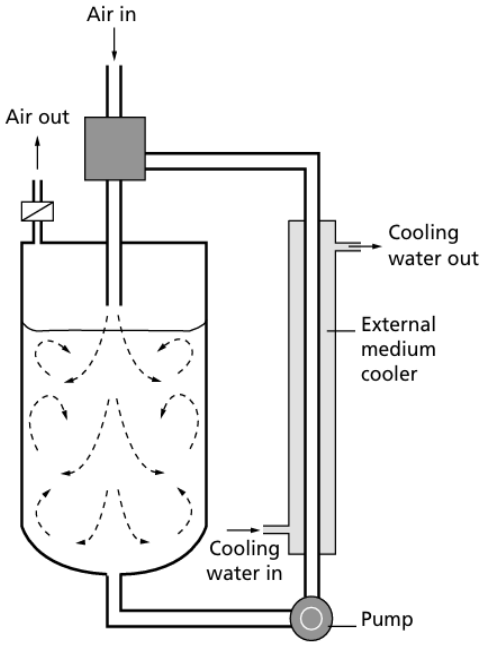


Fig. 6.3 A deep-jet fermenter.

5. Heat transfer

5.1 Sources of heat

- **Microbial metabolism:** Exothermic reactions
- **Mechanical agitation:** Friction and turbulence

5.2 Heat removal or addition

- Most fermentations require **cooling**
- Thermophilic fermentations may require **heating**
- Heat transfer is achieved via:
    - **External jacket** around the vessel
    - **Internal coils** (for larger vessels)
- No direct contact between cooling/heating fluid and fermentation medium

5.3 Sterilization use

The same jacket and coils are used to **sterilize the vessel** before inoculation by injecting pressurized steam.

5.4 Automatic temperature control

Cold or hot water is automatically circulated through the jacket/coils based on temperature sensor readings.

6. Mass transfer – aeration

6.1 Importance

Oxygen is **poorly soluble** in water (≈8 mg/L at 25°C). In aerobic fermentations, oxygen is often the **limiting substrate**.

6.2 Aeration system components

- **Air compressor** (oil and moisture removed)
- **Sterile filters** on both inlet and exhaust
- **Sparger** (located below the agitator in STRs)
- Typical airflow rate: 0.5–1.0 **vvm** (volumes of air per volume of medium per minute)

6.3 Factors affecting oxygen transfer rate (OTR)

1. Temperature and pressure
2. Bubble size (smaller = better, but smaller pores clog more easily)
3. Medium composition (solutes and surfactants affect diffusion)
4. Airflow rate (vvm)
5. Sparger type
6. Agitation speed

Key equation: $OTR = KLa \cdot (C^* - CL)$

Where:

- OTR = oxygen transfer rate (mmol/L/h)
- KLa = volumetric mass transfer coefficient (h^{-1})
- C^* = saturated dissolved oxygen concentration (mmol/L)
- CL = actual dissolved oxygen concentration at time t (mmol/L)

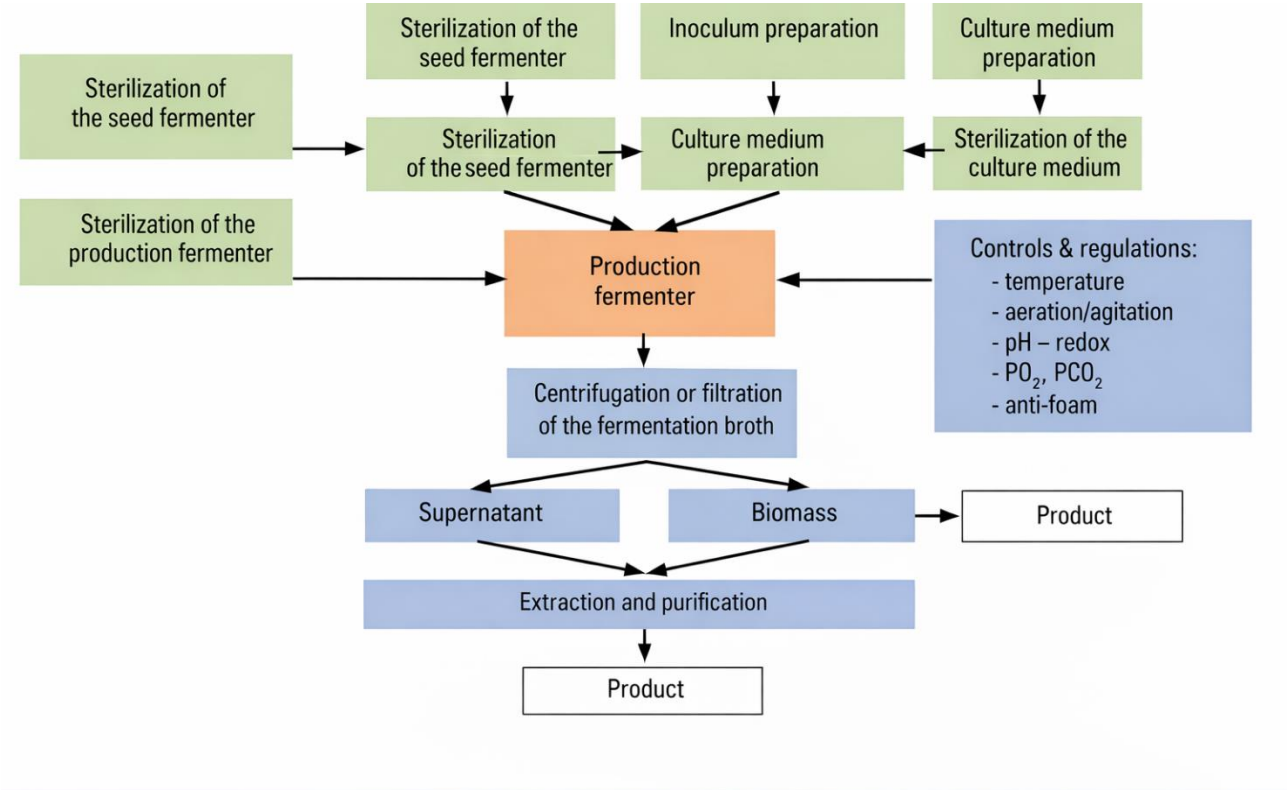
KLa is the standard parameter for comparing oxygen transfer efficiency between fermenters.

7. Operation of an industrial fermentation

In industrial microbiology, fermentation refers to the unit operation that produces biomass or bioconversion products through the cultivation of microorganisms.

There are five important steps in any fermentation process:

1. Preparation of the culture medium;
2. Sterilization of the bioreactor, its equipment, and the culture medium;
3. Preparation of the inoculum;
4. Production in the bioreactor;
5. Product extraction and purification.



Steps of the fermentation process

8. Fermenter control and monitoring

Fermentation systems must be efficiently controlled in order to optimize productivity and product yield, and ensure reproducibility. The key physical and chemical parameters involved largely depend on the bioreactor, its mode of operation and the microorganism being used.

8.1 Control loop principle

Sensor → Controller → Actuator → Feedback → Maintain setpoint

8.2 Parameters and sensors

Parameter	Sensor type	Control action
pH	pH electrode	Add acid or base (e.g., NH_4OH)
Temperature	Resistance thermometer, thermistor	Circulate hot/cold water
Dissolved O_2	Polarographic or galvanic probe	Change agitation, aeration, or enrich with O_2
Dissolved CO_2	CO_2 electrode	Adjust aeration
Foam	Foam probe (conductivity)	Add antifoam or activate mechanical breaker
Pressure	Pressure gauge	Safety valves, burst discs

8.3 Data logging and modeling

All sensor data is sent to a computer to:

- Calculate biomass and product formation
- Determine OTR and CO_2 transfer rates
- Track nutrient utilization and power usage
- Build mathematical models for future fermentations

8.4 Calibration

Control systems must be calibrated when first installed and regularly checked according to **Good Manufacturing Practices (GMP)**.

9. Bioreactor design criteria

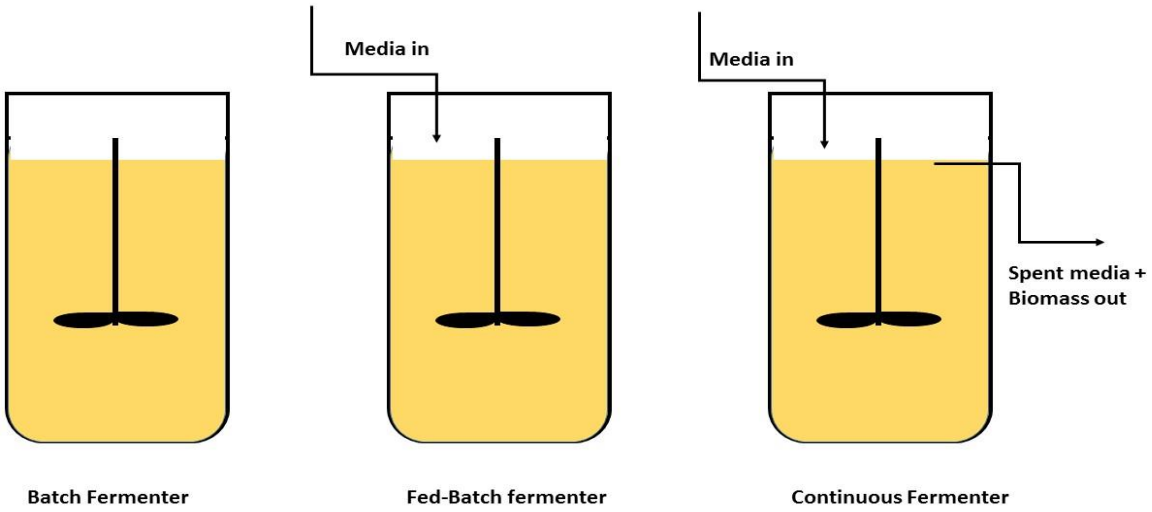
Bioreactors are designed to ensure **5 main functions**:

1. Maintaining sterility
2. Good mass transfer
3. Good heat transfer
4. Parameter monitoring and control management
5. Cleanability

10. Operating Modes

10.1 Batch fermentation

Batch fermentation is a closed system where no nutrients or components are added after inoculation, aside from acid, base, or air for pH and oxygen control. It typically follows a four-phase growth profile: lag, exponential (log), stationary, and death. This method offers advantages such as lower capital investment and the ability to be easily terminated and restarted in the event of contamination. However, it also presents several drawbacks, including significant downtime for cleaning, sterilizing, refilling, and cooling between batches; batch-to-batch variability; and the fact that only a fraction of the overall cycle is truly productive. Common applications include the production of alcoholic beverages, most amino acids, enzymes, and organic acids.



10.2 Fed-batch fermentation

Fed-batch fermentation is a semi-closed system in which nutrients are added during the process, either continuously, intermittently, or as a single supplement, without removing the culture broth. This approach extends the product formation phase, avoids catabolic repression caused by rapidly metabolized substrates such as glucose, and helps reduce problems related to toxicity or high viscosity. Common examples include the production of baker's yeast and penicillin. A variation of this method, known as fed-batch with cell recycle, retains and reuses biomass to enhance productivity and is typically applied in ethanol production or wastewater treatment processes.

10.3 Continuous fermentation (chemostat)

Continuous fermentation, also known as a chemostat, is an open system where fresh medium is added continuously while the culture is removed at the same rate, maintaining a constant volume. This setup reaches a steady state, meaning both the limiting nutrient concentration and cell number remain constant over time. The main advantages include higher productivity, reduced downtime, and lower operating costs. However, disadvantages include a higher initial capital investment, the challenge of maintaining sterility over extended periods (weeks), and the risk of genetic instability, where low-yielding mutants may outgrow the high-yielding strain. Common applications include biomass production, fuel ethanol, and effluent treatment.

11. Sterilization

11.1 Air sterilization

- **Inlet air:** Passed through sterile filters (glass fiber, mineral fiber, PTFE, PVC)
- **Exhaust air:** Filtered to prevent environmental contamination; for pathogens, also incinerated (dry heat)

11.2 Media and vessel sterilization

Small scale (1–5 L)

- Autoclave: 121°C for 15 minutes
- Care: Vent to avoid pressure build-up without contaminating contents

Pilot and industrial scale

- Steam under pressure (121°C or higher, longer time)
- Steam can be injected into the jacket, internal coils, or directly into the headspace

12. Solid-Substrate Fermentations (SSF)

Growth of microorganisms on solid, organic materials with **little or no free water** (water activity *Aw* typically around 0.7).

12.1 Traditional and modern applications

Traditional	Modern
Tempeh, sufu	Enzymes
Cheese, mushrooms	Organic acids
Compost, silage	Ethanol
Oriental fermented foods	Spore production (e.g., *Coniothyrium minitans*)

12.2 Microorganisms used

- Filamentous fungi (most common)
- Some bacteria (Actinomycetes, *Bacillus*)
- Must tolerate low water activity

12.3 Process steps

1. Substrate pretreatment (mechanical, chemical, or biological)
2. Hydrolysis of polymers (polysaccharides, proteins)
3. Utilization of hydrolysis products
4. Separation and purification of end-products

12.4 Environmental parameters

Parameter	Effect
Water activity (Aw)	Too low → substrate inaccessible, no growth; too high → porosity reduced, oxygen diffusion limited
Temperature	Major problem; controlled by aeration and/or agitation
Aeration	Oxygen dissolves in water film around particles; excess water fills void spaces and blocks oxygen

11.5 Bioreactors for SSF

Type	Description	Capacity / use
Rotating drum	Cylinder on rollers, rotated	~100 L; max 30% fill
Tray fermenter	Substrate on trays (few cm deep), stacked in a chamber	Up to 150 m^3
Bed system	Deep bed (up to 1 m), humidified air forced from below	Commercial koji
Column bioreactor	Glass/plastic column, jacketed, loosely packed	Organic acids, ethanol
Fluidized bed	Continuous agitation with forced air	Animal feed biomass

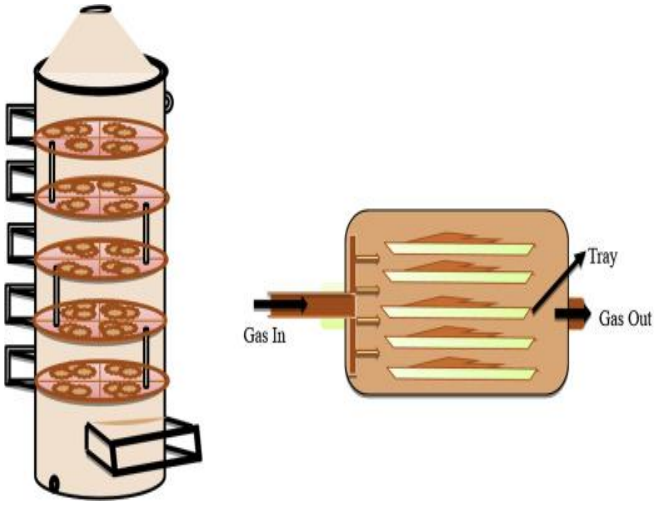


Fig. Tray fermenter

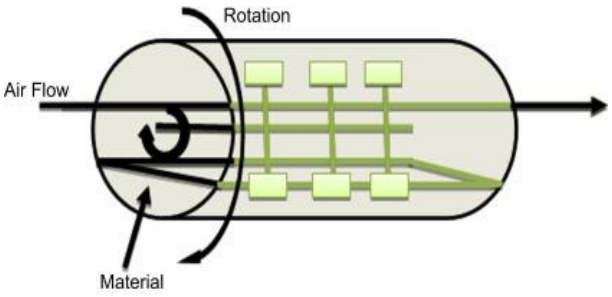


Fig. Schematic representation of rotating-drum and stirred-drum bioreactors.

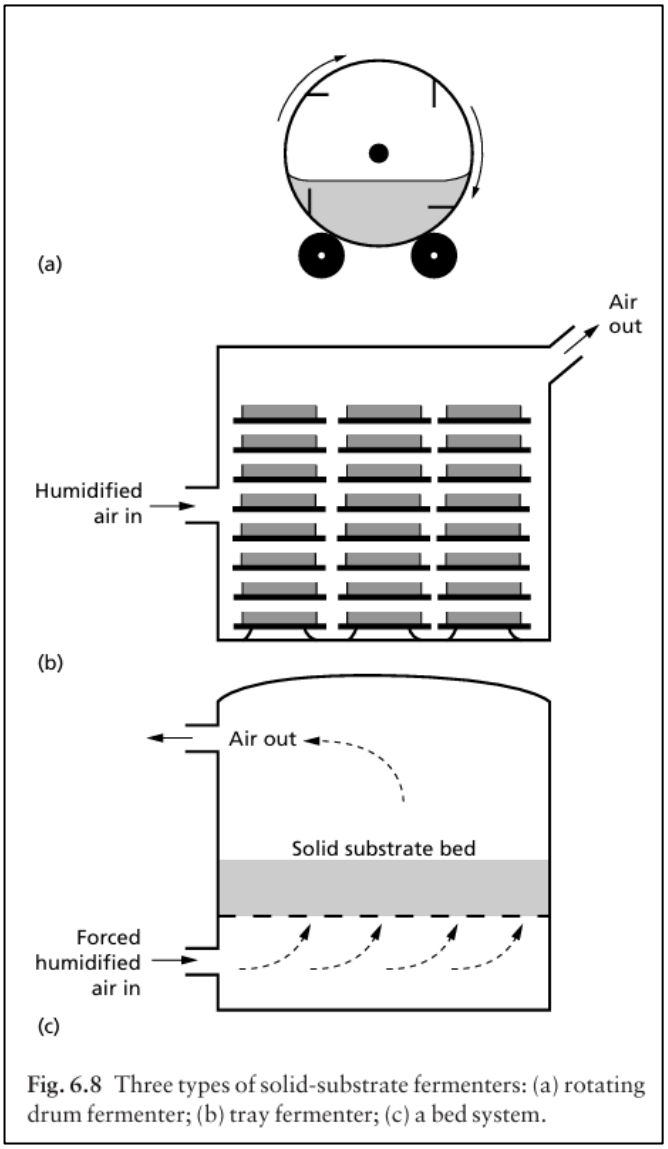
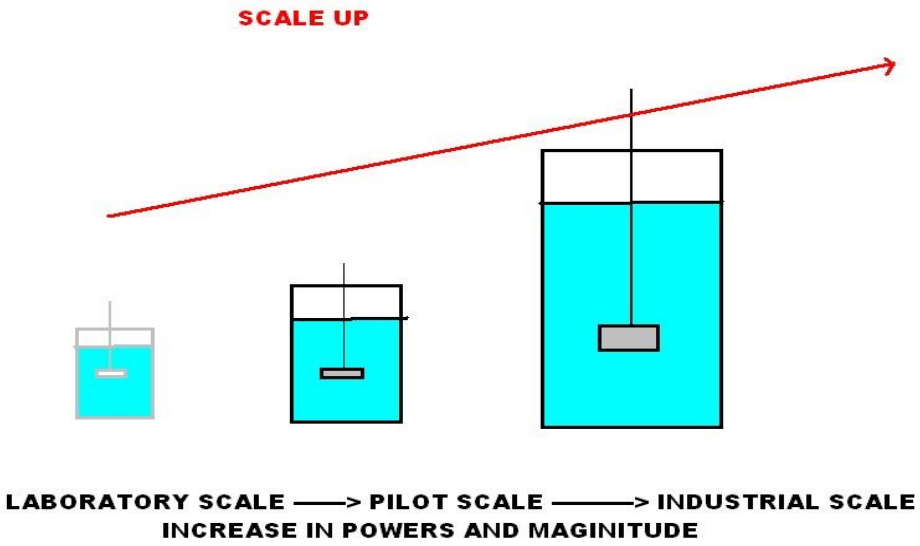


Fig. 6.8 Three types of solid-substrate fermenters: (a) rotating drum fermenter; (b) tray fermenter; (c) a bed system.

13. Fermentation process development (Scale-Up)



13.1 Typical scale-up pathway

Optimization of product yield in the labo ratory is followed by process scale-up; first to pilot scale of 10–100L and finally to industrial scale of 1000 100000L, or more, depending upon the specific process.

Laboratory (1–10 L) → Pilot (10–100 L) → Industrial (1000–100,000+ L)

To ensure successful scale-up, various physicochemical parameters are analyzed and modified during each step, because the physicochemical and enzymatic reactions of microbial cells occurring inside the bioreactor vary with the reactor volume. The goal is to achieve the same yield despite the increase in culture volume.

13.2 Parameters that can be fixed during scale-up (choose one)

- Height–diameter ratio (aspect ratio)
- Power input per unit volume (W/m^3)
- *KLa* (oxygen transfer coefficient)
- Dissolved oxygen level
- Impeller tip speed (to maintain similar shear forces)