به نام خدا

Fermentation systems

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Definition

- The nutrients Microbiologists use the term fermentation in two different contexts.
- First, in metabolism, fermentation refers to energy-generating processes where organic compounds act as both electron donor and acceptor .
- Second, in the context of industrial microbiology, the term also refers to the growth of large quantities of cells under aerobic or anaerobic conditions, within a vessel referred to as a fermenter or bioreactor.

- In this lecture we will concentrate on fermenters used in traditional microbial, plant and animal cell culture
- However with the advent of recombinant DNA technology alternate systems for producing specific cell products are now available…vaccines can be produced in sheep's milk or in fruit (e.g. biosynthesis of malaria vaccine in bananas)

What is a Fermentor?

- **Vessel or tank in which whole cells or cell-free enzymes transform raw materials into biochemical products and/or less undesirable by-products**

- **Also termed a Bioreactor**

Fermentor – Basic Function

The basic function of a fermentor is to provide a suitable environment in which an organism can efficiently produce a target product that may be
- cell biomass,
- a metabolite,
- or bioconversion product.

What is a Fermentor?

فرمنتورهای آزمایشگاهی

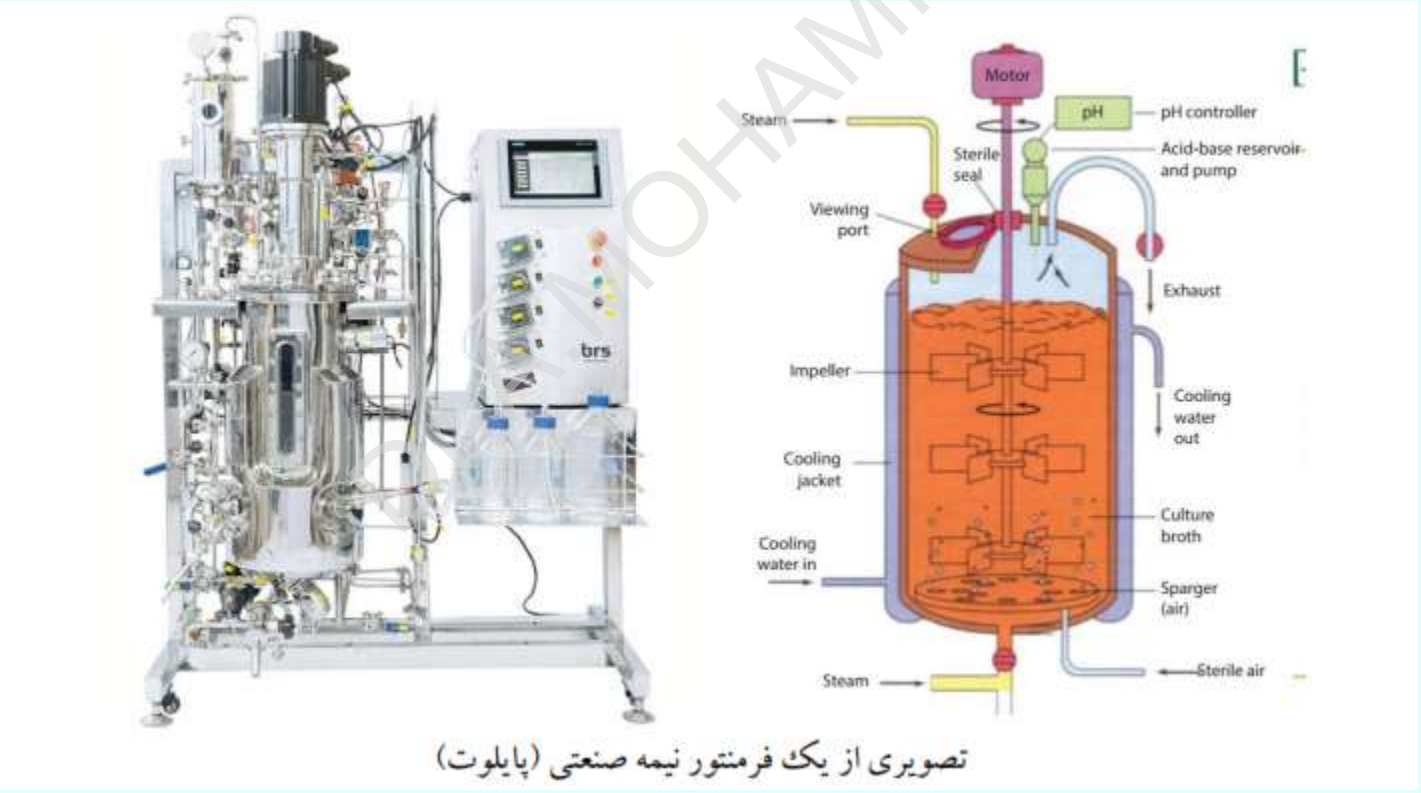
کوچکترین نوع فرمنتور بوده که دارای ظرفیت محدودی است؛ یعنی ۱ تا ۱۵ لیتر. به طور عمده این نوع از فرمنتورها به منظور تحقیق و توسعه استفاده می شوند. فرمنتورهای مقیاس آزمایشگاهی برای تعیین شرایط مطلوب رشد و بیوسنتز میکروارگانیسم ها بکار می رود.

تصویری از یک فرمنتور آزمایشگاهی

What is a Fermentor?

فرمنتورهای نیمه صنعتی

این فرمنتورها دارای اندازه متوسطی هستند و برای مطالعات تخمیر در مقیاس بزرگ مورد استفاده قرار می گیرند. حداقل و حداکثر ظرفیت آنها به ترتیب، ۹۴ و ۷۵۷۰ لیتر است.



تصویری از یک فرمنتور نیمه صنعتی (پایلوت)

What is a Fermentor?

فرمنتورهای صنعتی

این نوع از فرمنتورها برای تولید مقادیر بالا محصولات مهم تخمیری در صنایع مورد استفاده قرار می گیرد. ظرفیت آنها ۳۷۸۵۴,۱۲-۳۷۸۵۴۱,۱۸ لیتر است (شکل).

What is a Fermentor?

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تصویری از فرمنتورهای صنعتی

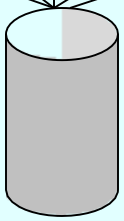
جدول ۱. تفاوت های بین راکتور و فرمنتور

تفاوت ها	فرمنتور	بیوراکتور
اندازه	کوچک، تا ۲ لیتر	بزرگ، از چند لیتر تا چندین مترمکعب
ماهیت	حساس نیستند، زیرا سلول های باکتریایی دارای دیواره سلولی قدرتمندی هستند	حساس هستند، زیرا سلول های پستاندار شکننده هستند و غشای سلولی حساس به برش دارند
سرعت تولید	سریع به دلیل سرعت رشد بالای میکروب ها	آرام به دلیل دو برابر شدن سلول های پستانداران در هر ۲۴ ساعت
مصرف اکسیژن	زیاد	کم
آلودگی	عدم تهدید ویروسی در سلولهای میکروبی لذا عدم نیاز به غیر فعال کردن و یا حذف	وجود تهدید، نیاز به غیر فعال کردن و یا حذف
سترون سازی	سترون سازی کامل	نبود سترون سازی

Biotechnological processing

- Types of Fermentation
- Process Design
- Fermenter Design
  - Performance
  - Optimisation
  - Construction
  - Configuration
  - Control

Fermenter design involves cooperation in Microbiology, Biochemistry, Chemical Engineering, Mechanical Engineering, Economics



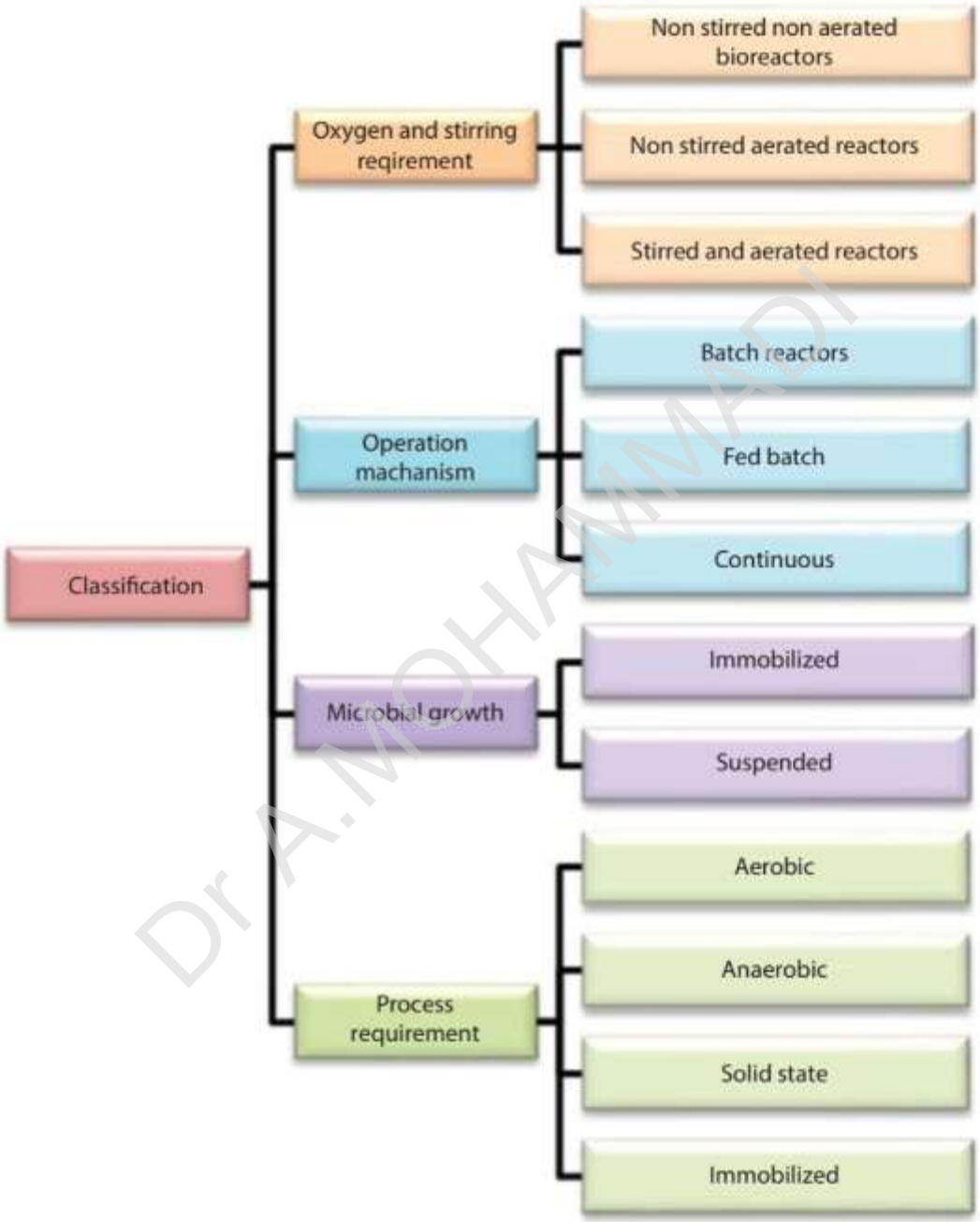
Fermenters range from simple stirred tanks to complex integrated systems involving varying levels of computer input.

Stirred Tank Reactor

What is a Fermentor?

طبقه بندی بیوراکتور

در فرمنتورها و بیوراکتورها، فرآیندهای بیوشیمیایی مختلفی با کمک میکروب هایی مانند باکتری ها، قارچ ها، سلول های پستانداران و سیستم های سلول گیاهی برای تولید انواع محصولات بیولوژیکی به شکل محصول اولیه صورت می گیرد. آنها محیط محرک برای تولید متابولیت را فراهم می کنند. به طور عمده فرمنتور و بیوراکتور بر اساس نیاز به اکسیژن و همزدن، مکانیسم عمل، رشد میکروبی و نیازهای فرآیند مربوطه به چهار گروه اصلی تقسیم می شوند که در شکل نشان داده شده است. بیوراکتورهای غیر همزن و هوادهی نشده[1] برای تولیدات سنتی همانند شراب، پنیر و آبجو استفاده می شوند. در حالی که راکتورهای همزن دار هوادهی شده در فناوری های مدرن بکار گرفته می شوند.



طبقه بندی های مختلف بیوراکتور و فرمنتور

Classification

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			

Although solid-substrate fermentations are operated, most fermentations use liquid media, often referred to as broth, under aerobic or anaerobic conditions.
Some, like beer and wine fermentations, are nonstirred, non-aerated and are not operated aseptically, whereas many others are stirred, aerated and aseptic.

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

Classification

Fermentations are also broadly classified according to the organization of the biological phase, whether it is in suspension or in the form of a supported film

Table 6.2 Classification of industrial fermentations according to the organization of the biological phase

Suspended mode		Supported mode	
Individual cells	Flocs and aggregates	Fixed film	Films on fluidized supports
Cylindroconical vessels *Saccharomyces cerevisiae* (beer)	Activated sludge reactor mixed culture (waste-water treatment)	Trickling film generator acetic acid bacteria (vinegar)	Fluidized bed reactor mixed culture (waste-water treatment)
Airlift fermenter *Methylophilus methylotrophus* (biomass)	Stirred tank reactor *Aspergillus niger* (citric acid production)	Trickle filters mixed culture (waste-water treatment)	Fluidized bed reactor animal cells (monoclonal antibodies)
Stirred tank reactor *Bacillus subtilis* (enzymes)	Stirred tank reactor *Penicillium chrysogenum* (penicillin)	Hollow fibre fermenter animal cells (monoclonal antibodies)	

Fermenter design and construction

- The performance of any fermenter depends on many factors, but the key physical and chemical parameters that must be controlled are agitation rate, oxygen transfer, pH, temperature and foam production.

- Low value products that include many of the traditional fermentation products, such as alcoholic beverages, are usually produced using relatively simple fermenters and may not operate under aseptic conditions.

- Other fermentations do not involve pure culture inoculum and actively encourage the development of indigenous microorganisms, e.g. some food fermentations and waste-water treatment.

- Conversely, fermentations producing high value, relatively low volume products, especially pharmaceuticals, invariably demand more elaborate systems and operate under strict aseptic conditions.

Fermenter design and construction

- **Small** fermentation vessels of a few litres capacity are constructed from glass and/or stainless steel.
- **Pilot scale** and many production vessels are normally made of stainless steel with polished internal surfaces,
- whereas **very large** fermenters are often constructed from mild steel lined with glass or plastic, in order to reduce the cost.
- If aseptic operation is required, all associated pipelines transporting air, inoculum and nutrients for the fermentation need to be sterilizable, usually by steam.

Fermenter design and construction

- The design rules for an aseptic bioreactor demand that there is no direct contact between the sterile and non-sterile sections to eliminate microbial contamination.

- Any connections to the fermenter should be suitable for steam treatment to kill any resident microorganisms and systems must be designed to allow aseptic inoculation, sampling and harvesting.

- Every individual part should be easily maintained, cleaned and independently steam sterilizable, particularly valves.

- Most vessel cleaning operations are now automated using spray jets, which are located within the vessels. They efficiently disperse cleaning fluids and this cleaning mechanism is referred to as cleaning-in-place (CIP)

Control of chemical and physical conditions

- **Intensive properties** (cannot be balanced) - temperature, concentration, pressure, specific heat
- **Extrinsive properties** (can be balanced) - mass, volume, entropy and energy
- For example, if 10g of water at 30°C is added to 35g of water at 30°C, the resulting water has a temperature (intensive property) of 30°C not 60°C, but the mass of water (extrinsive property) is additive at 45g.
- Mass and energy levels should balance at the start and finish of fermentations.
- Combining this with determination of thermodynamic properties and rate equations we can build computer and mathematical models to control processes

Basic Fermenter Design Criteria

(i). Nature of microbial (or mammalian, plant tissue) cell;

(a) Hydrodynamic characteristics

(b) Mass and Heat Transfer

(c) Kinetics

(d) Genotype and Phenotype

(ii). Environmental Control and Monitoring of the process;

(a) pH, temperature, dissolved oxygen etc.

(b) Asepsis and avoidance of contamination

(iii). Process factors;

(a) Effect on other unit operations

(b) Economics

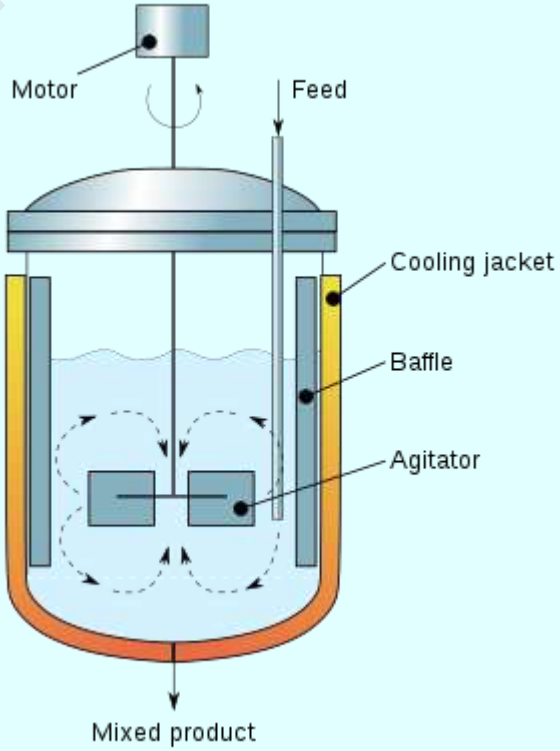
(c) Potential for scale-up

Agitation

- Efficient mixing is particularly important for oxygen transfer in aerobic fermentations, as microorganisms can take up oxygen only from the liquid phase.
- Transfer into liquid from the gaseous phase is enhanced by agitation.
- It prolongs retention of air bubbles in suspension, reduces bubble size to increase the surface area for oxygen transfer, prevents bubble coalescence and decreases the film thickness at the gas-liquid interface.
- Fermenter agitation requires a substantial input of energy and there are three principal mechanisms that may be used:
- 1- Stirred tank reactors (STRs)
- 2- Pneumatic systems
- 3- Hydrodynamic mechanisms

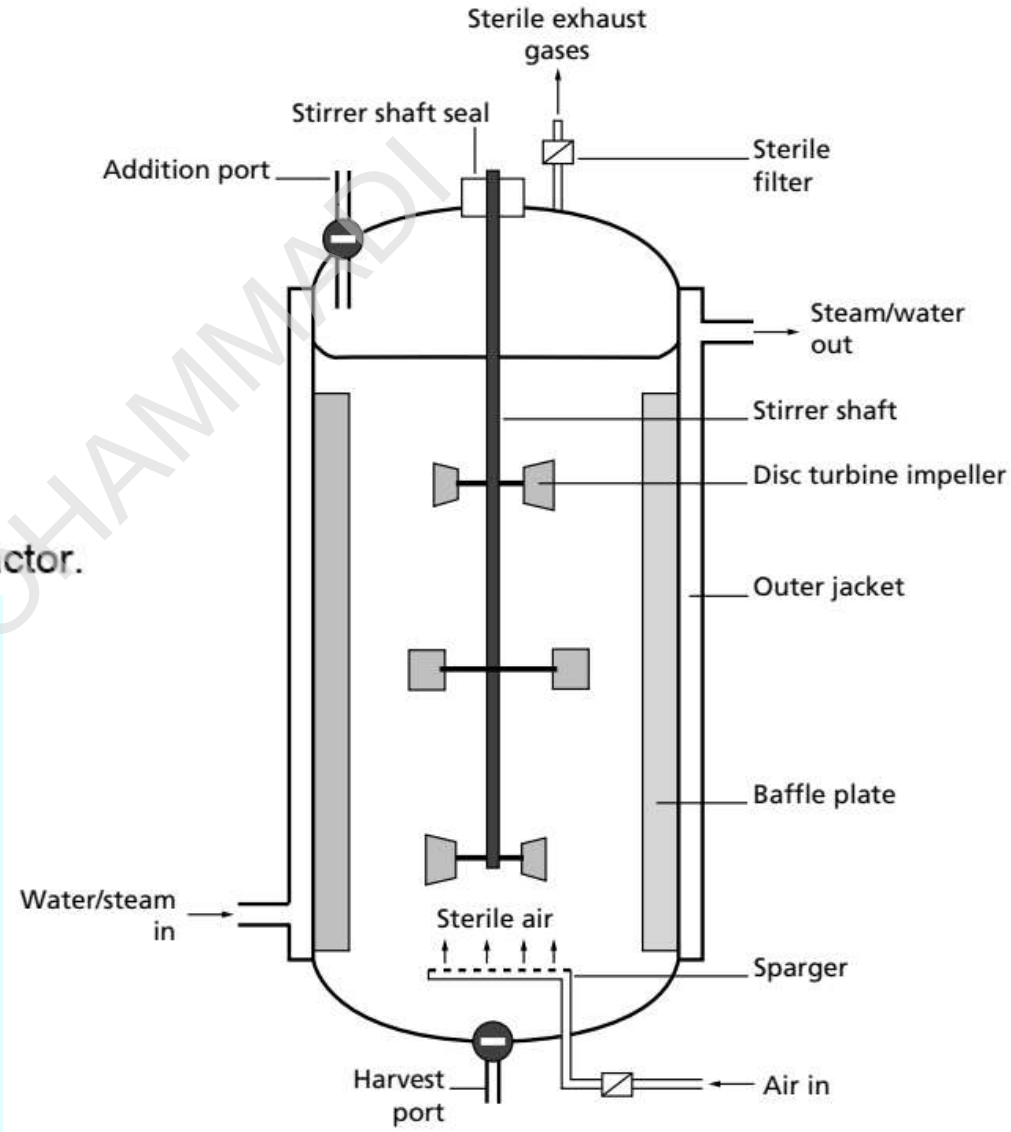
1- Stirred tank reactors (STRs)

- • Most commonly fermenter used
- • Made from stainless steel when over 20 Litres
- • Baffles prevent a large central vortex
- • Also used to carry coolants in large systems



1- Stirred tank reactors (STRs)

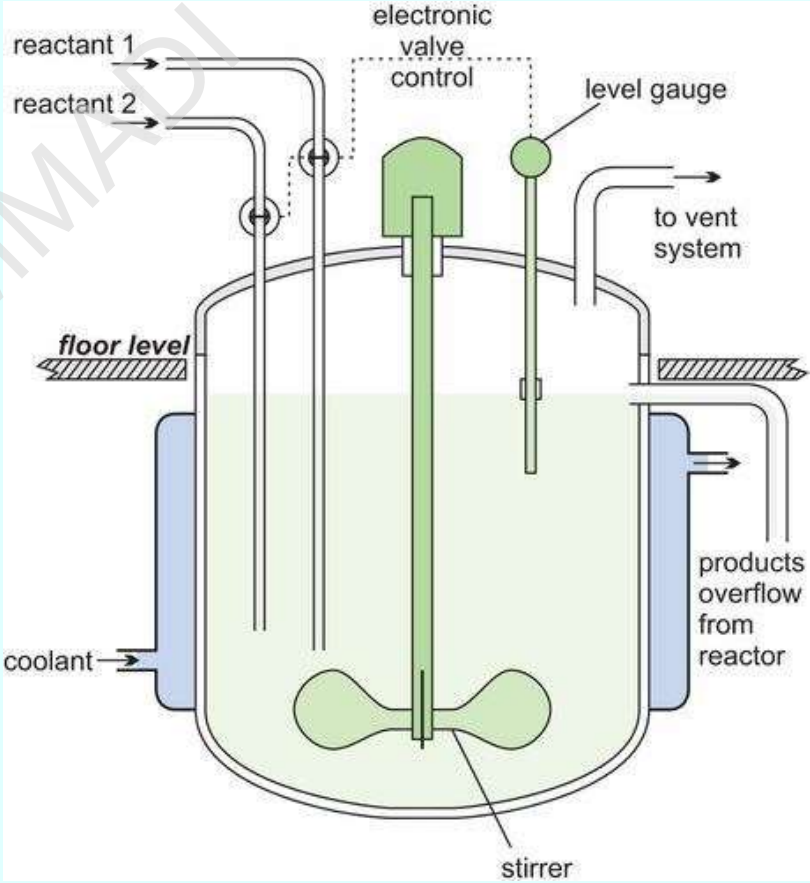
- An agitator system
- An oxygen delivery system
- A foam control system
- A temperature control system
- A pH control system
- Sampling ports
- A cleaning and sterilization system.
- A sump and dump line for emptying of the reactor.



- **The effectiveness of agitation depends upon the design of the impeller blades, speed of agitation and the depth of liquid.**
- **Most STRs have height–diameter aspect ratios of 3:1 or 4:1.**

1- Stirred tank reactors (STRs)

- **STRs must create high turbulence to maintain transfer rates, but this also generates considerable shear force that is detrimental to certain cells.**
- **For instance, many animal and plant cells are shear sensitive and excessive stirring may result in cell disruption.**
- **In these cases STRs may be unsuitable without modification, and airlift or supported biofilm reactors may be preferred**



2 Pneumatic systems

- **Pneumatic systems, such as airlift fermenters, have no moving parts and use the expansion of compressed gas to bring about the mixing.**

- **These systems have lower energy requirements and create less shear than STRs.**

- **Liquid movement is initiated by the injection of compressed air at the bottom of the internal or external riser column and the air bubbles expand in the riser causing the upward movement of liquid and initiating its cycling within the fermenter.**

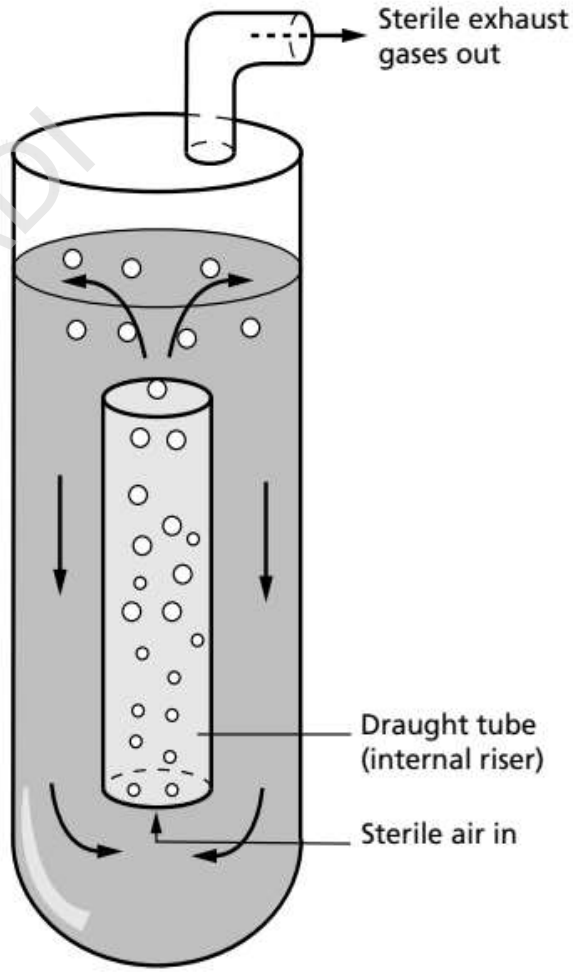
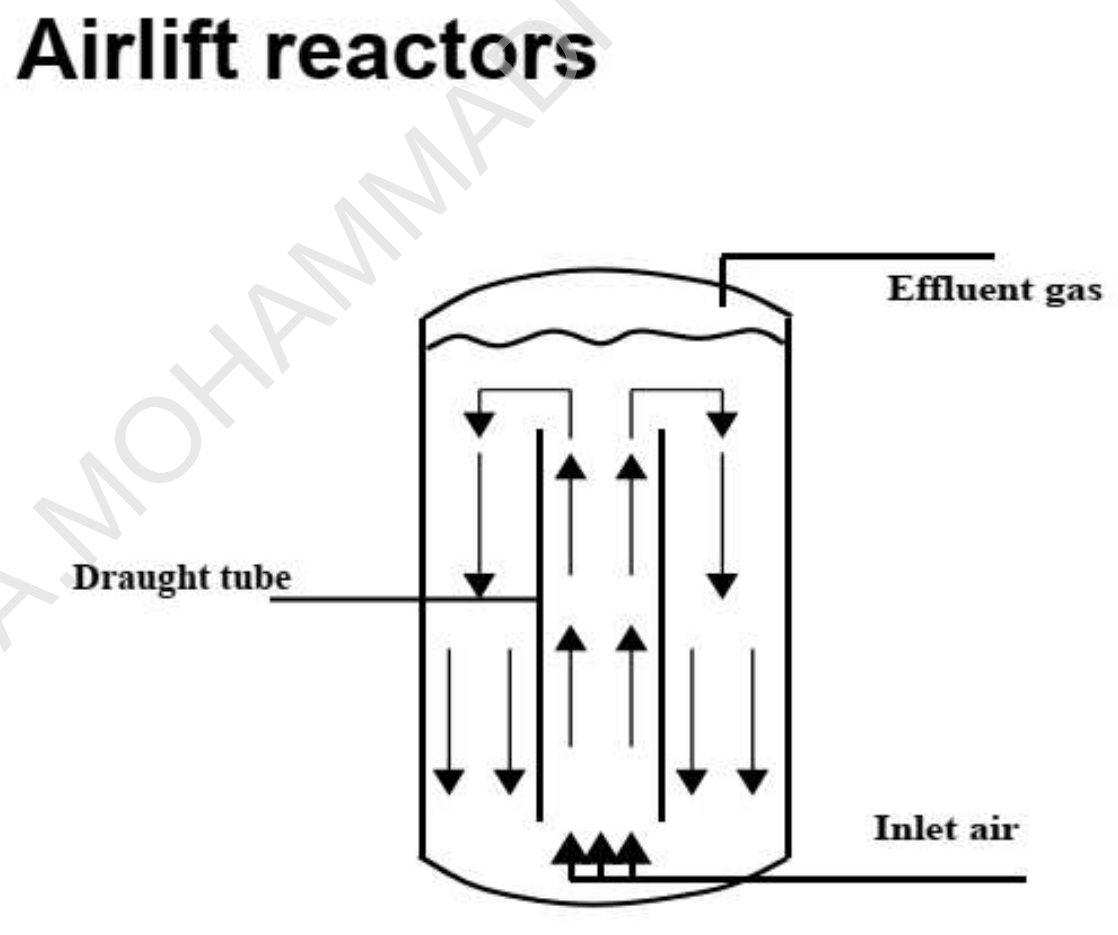


Fig. 6.2 A diagram illustrating the principle of an airlift fermenter.

- Even large fermenters do not require internal cooling coils as a jacket can normally provide sufficient heat transfer, due to the rapid movement of fluid within the vessel



3 Hydrodynamic mechanisms

- **It use liquid kinetic energy to mix the fermenter contents, which is achieved by using an external liquid pump for external circulation and reinjection, e.g. deep-jet fermenters**

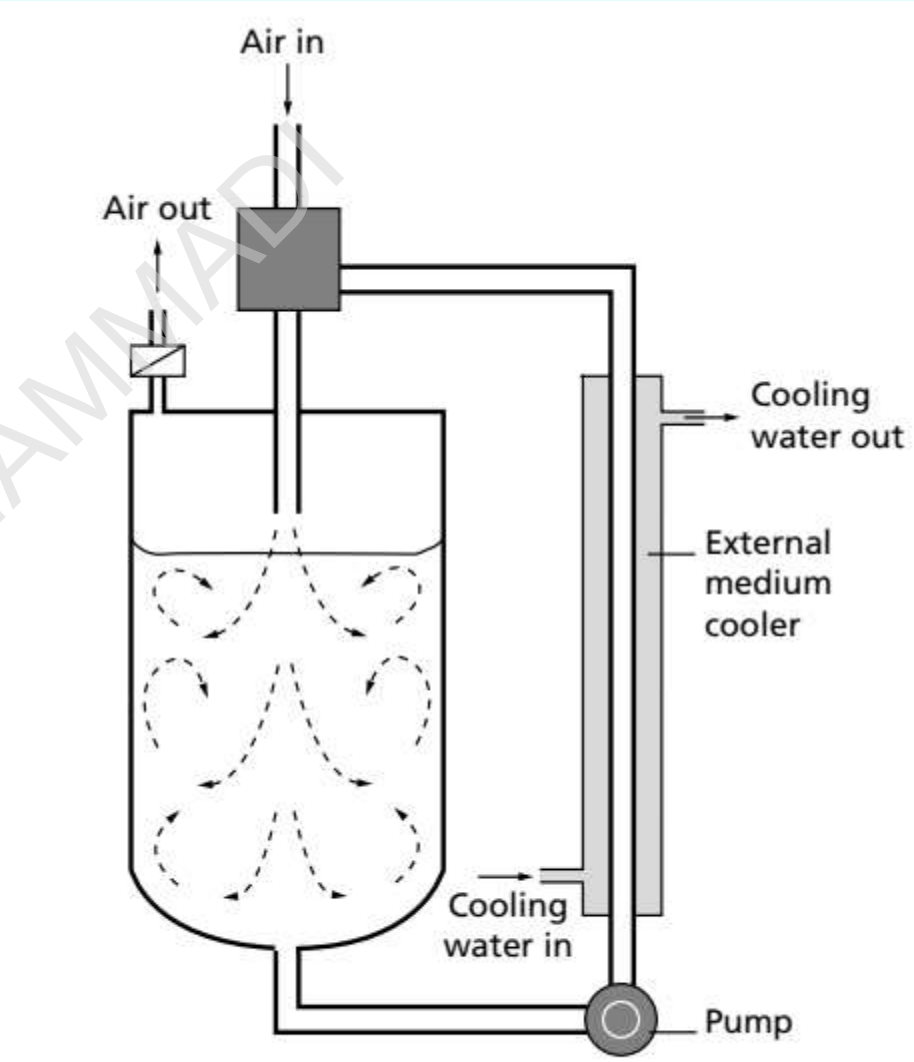


Fig. 6.3 A deep-jet fermenter.

Solid-substrate fermentations

- It involves the growth of microorganisms on solid, normally organic, materials in the absence or near absence of free water.
- The substrates used are often cereal grains, bran, legumes and lignocellulosic materials, such as straw, wood chippings, etc.
- Traditional processes are largely food fermentations producing oriental tempeh and sufu, cheeses and mushrooms; along with compost and silage making.
- In addition, enzymes, organic acids and ethanol are now produced by SSF, particularly in areas where modern fermentation equipment is unavailable.
- Most fungi do not form spores in submerged fermentations, but sporulation is often accomplished in SSF

Solid-substrate fermentations

Table 6.4 Advantages and disadvantages of solid-substrate fermentations

Advantages	Disadvantages
Potentially provide superior productivity	Slower microbial growth
Low-cost media	Problems with heat build-up
Simple technology	Bacterial contamination can be problematic
Low capital costs	Difficulties often encountered on scale-up
Reduced energy requirements	Substrate moisture level difficult to control
Low waste-water output	
No problems with foaming	

Solid-substrate fermentations

- **It is normally multistep processes, involving:**

1 pretreatment of a substrate that often requires mechanical, chemical or biological processing;
2 hydrolysis of primarily polymeric substrates, e.g. polysaccharides and proteins;
3 utilization of hydrolysis products; and
4 separation and purification of end-products.