Chapter VI. Industrial production processes

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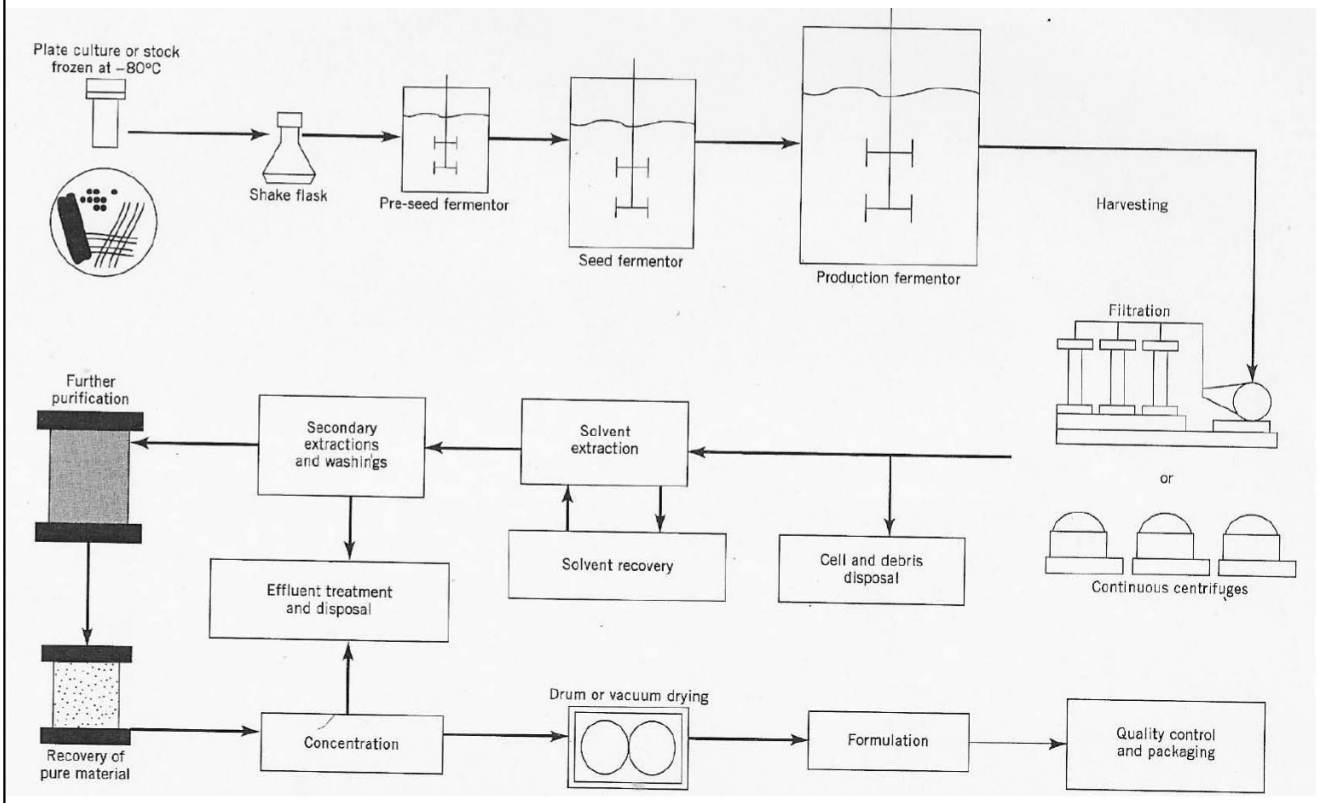


Fig. 1.2 Flowchart of the Production Process in a Typical Industrial Microbiology Establishment

- In many industrial processes, microorganisms must be cultivated in specific environments with rigorous control of conditions such as temperature, aeration, and nutrient supply.
- This requires specialized equipment, such as fermenters or bioreactors, which are equipped with control systems to manage various parameters that limit microbial growth and production.

« Fermentation »

- Although 'fermentation' technically refers to anaerobic respiration, as defined by Pasteur, the term is widely used in industry to refer more broadly to the :

«process of cultivating cells and conducting bioconversion reactions, whether aerobic or anaerobic»

- Fermentation is a stage in an industrial process and part of a series of unit operations aimed at valorizing biomass, its components, or the products of bioconversion.

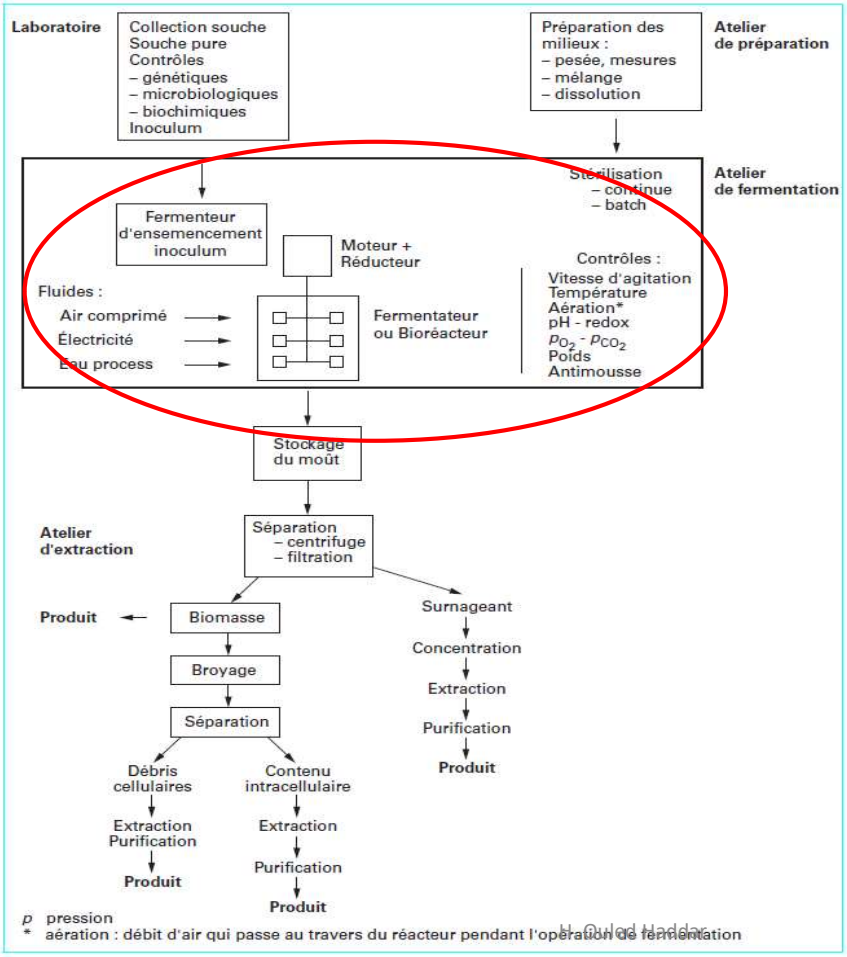


Figure 1 – Fermentation et environnement : schéma de principe des procédés fermentaires

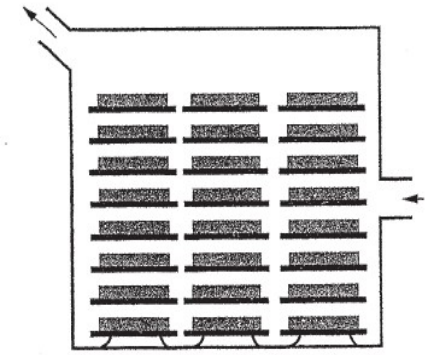
Different cultivation methods in IM

1. Fermentation in solid media (low a_w)

Simple, inexpensive, limited process control

- **Applications**

Maintenance of strains, production of enzymes, composting, cheese, bread, mushrooms…

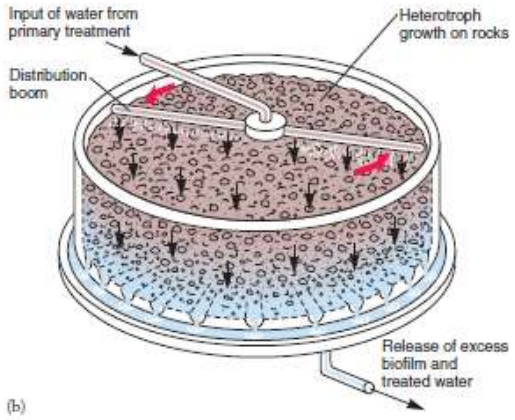


2. Biofilms

Different types of bioreactors, rotating disks, biofilters, fixed bed reactors

- **Applications**

Wastewater treatment, vinegar…

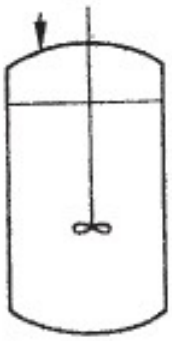


3. Batch culture (discontinuous):

Different types of reactors, rotary agitation, air lift, process control is possible

- **Applications**

Antibiotics, solvents, acids…



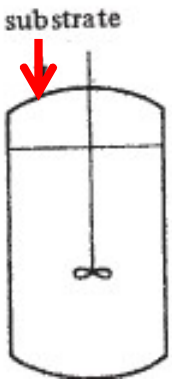
Batch fermenter (BF)

4.Fed-batch culture (fed batch)

Regulation of metabolic repressions (glucose)

- **Applications**

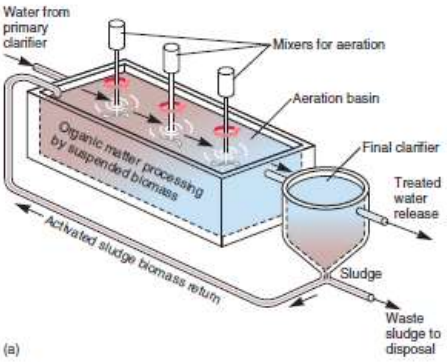
Baker's yeast



Fed_batch fermenter FBF

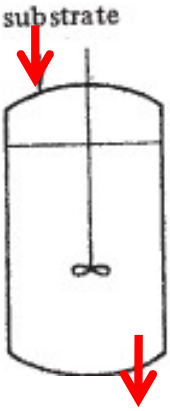
5. Continuous culture

Excellent for kinetics studies, high cost, contamination issues



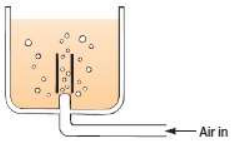
- **Applications**

Single Cell proteins (SCP), wastewater treatment..

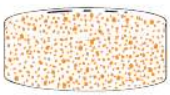


Continuous fermenter

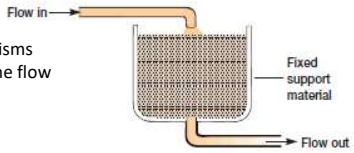
(a) Fermenter with pneumatic agitation (air-lift): the difference in density of the gas bubbles entrained in the medium produces a circulation of liquide



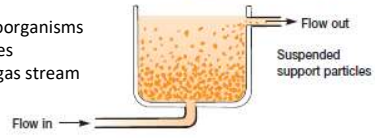
(b) Solid state fermentation: culture development in the absence of added free water



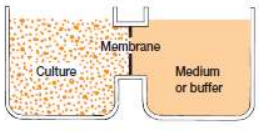
(c) Fixed bed reactor: microorganisms grow on solid support surfaces; the flow can be up or down



(d) Fluidized bed reactor: microorganisms grow on the surfaces of particles suspended in a rising liquid or gas stream



(e) Dialysis culture unit: The waste diffuses outwards from the culture; substrates diffuse across the membrane towards the culture



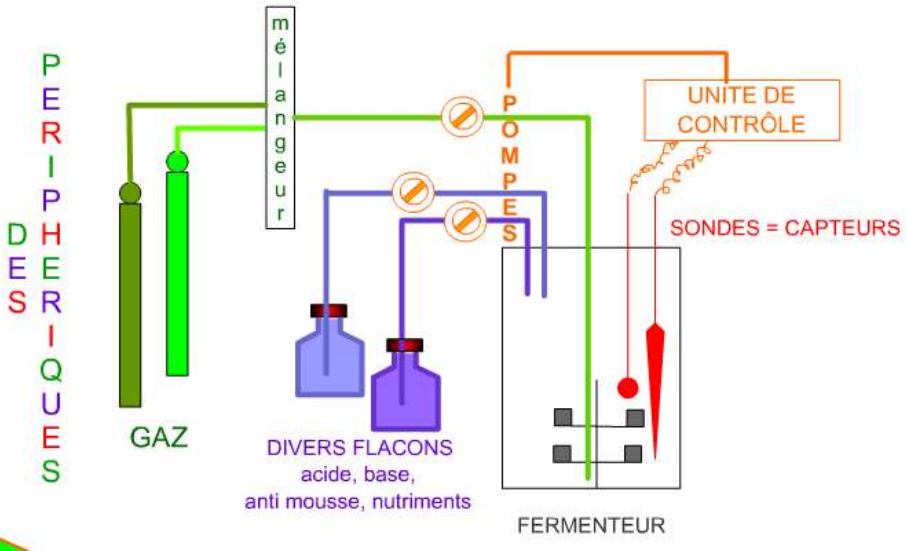
(f) Continuous culture unit (chemostat): entry of medium and rejection of excess medium and cells

Figure 42.8 Alternate Methods for Mass Culture. In addition to stirred fermenters, other methods can be used to culture microorganisms in industrial processes. In many cases these alternate approaches will have lower operating costs and can provide specialized growth conditions needed for product synthesis.

Fermenters

- A fermenter is a container designed for the growth of microorganisms under controlled conditions.
- It prevents contamination while providing optimal pH, temperature, oxygen, and other environmental factors to maximize the production of desired products.
- In chemical industry, containers where reactions occur are called reactors, so fermenters are also known as bioreactors

- 1 to 20 liters on a laboratory scale
- 100,000 to 500,000 liters for industrial fermenters.
- Between these two extremes, we find pilot fermenters (20 to 1000 liters).

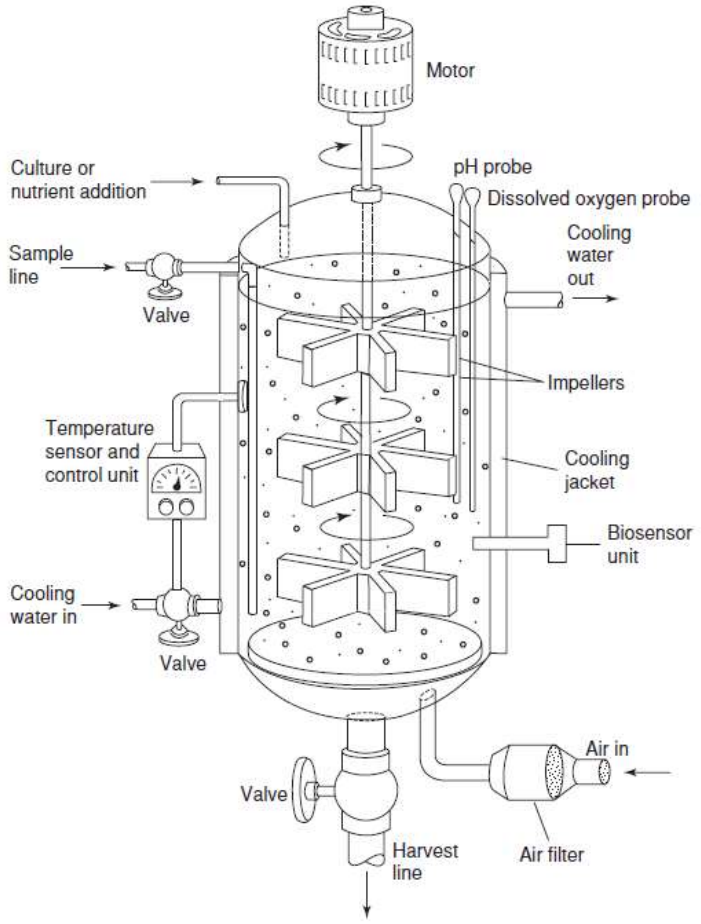


Qu'est ce qu'une UNITE DE FERMENTATION ?

Un fermenteur + des périphériques

Cet ensemble peut être contrôlé et régulé afin de suivre et éventuellement optimiser les conditions de culture

Composition



Materials

- Solid stainless steel, resists acidity, temperature, hydrostatic pressure, rigorous control of culture conditions (industrial fermenters, pilots)

- Pyrex glass (laboratory fermenters)
- Wood (open fermenters)
- Concrete (open fermenters)

Measurements, control and regulation

Common measurements include:

— temperature;
— aeration (i.e. volume of air injected into the reactor per unit of time);
— stirring speed
— pH;
— pressure ;
— foam detection;
— dissolved oxygen;
— dissolved carbon dioxide;
— O_2 in the gas phase at the fermenter outlet;
— CO_2 in the gas phase at the fermenter outlet;
—mass.

More specific measures may be found:
— turbidimetry;
— filtration loop with online analysis of glucose-organic acids by HPLC (High Pressure Liquid Chromatography);
— methane and hydrogen (methanization).

Control

1. Temperature control:

This is a major criterion in fermenter design. Typically, it involves maintaining the fermentation temperature within ±1°C. Because fermentation reactions can be highly exothermic, this criterion requires careful consideration

Heat exchanger: double wall (double paroi) or coil system (serpentin)
Culture cooling=cold water
Heating=hot water
Combined systems

Internal heat exchanger

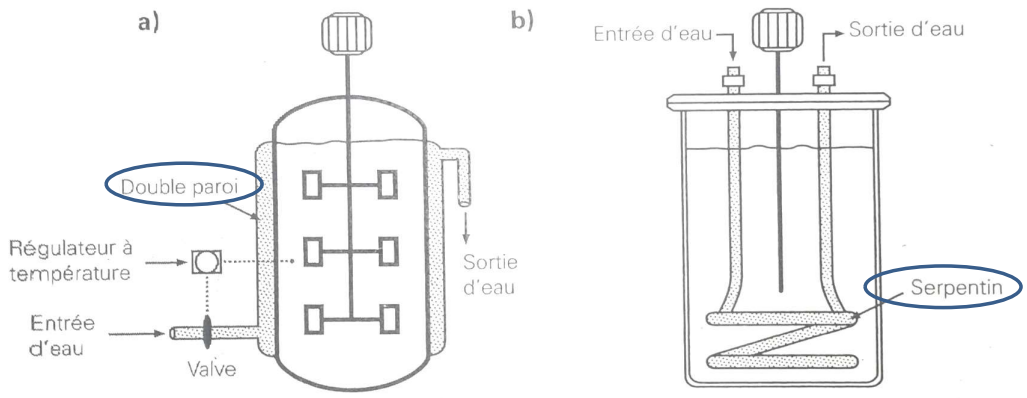


Figure 6.4 Systèmes de refroidissement des cuves de fermentation. (a) Système à double paroi, (b) système avec serpentin.

Remarks:

The double-wall (jacketed) system is convenient for steam sterilization but has limitations.

Beyond a certain tank volume, the internal surface area is insufficient for cooling the culture, a problem that can be resolved with a coil system.

For fermentations involving psychrophiles, a refrigerant (ethylene-glycol, ammonia, freon, CO_2..), rather than water, is circulated in the cooling system, though this has a higher operating cost.

The thermometer probe is placed in indirect contact with the culture, immersed in a steel well filled with a conductive liquid

Control

2. pH control:

During fermentation, the production of organic acids or alkaline substances can acidify or alkalize the medium.

An automated system injects a neutralizing agent (strong acid or base) as needed, controlled by a regulator connected to a probe (electrodes)

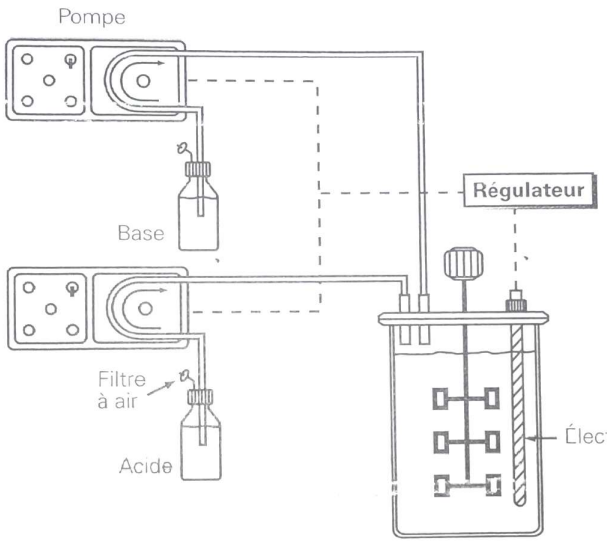


Figure 6.5 Schéma d'un système de contrôle du pH sur un réacteur de laboratoire. 194

Remarks:

The acids and bases used include sulfuric acid (H_2SO_4), hydrochloric acid (HCl), ammonium hydroxide (NH_4OH), and sodium hydroxide (NaOH).

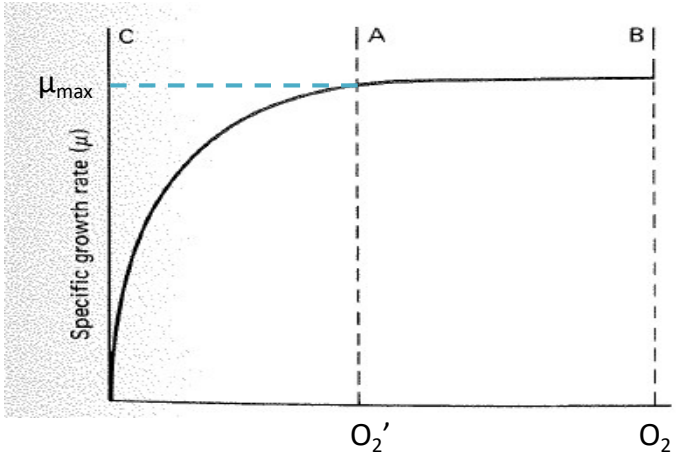
Standard pH probes are combined electrodes that measure potential differences proportional to the concentration of H^+ ions in the medium.

They are typically made of glass, pressurized, and resistant to sterilization temperatures

Control

3. Oxygenation Control

During aerobic fermentation, oxygen transfer in the bioreactor must be sufficient to keep the concentration of dissolved oxygen above the critical level, O_2'. This is achieved by controlling both aeration and agitation."



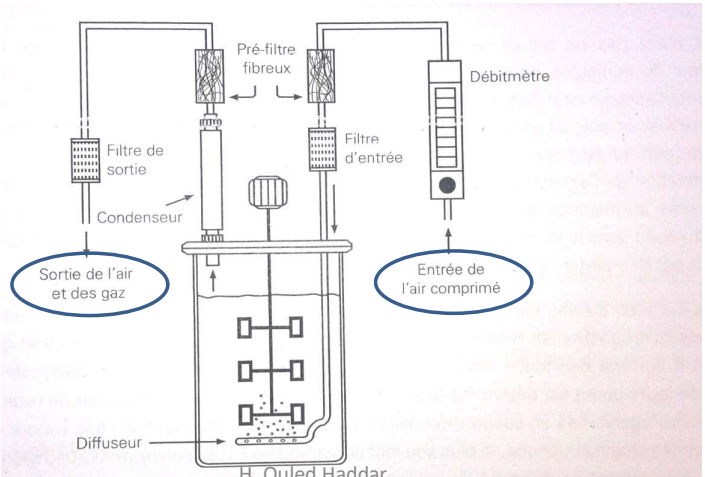
Monod relationship between specific growth rate and dissolved oxygen concentration in an aerobic culture.

3.1 Aeration

In industrial fermentation, oxygen is primarily supplied by air passing through the reactor, though it can be enriched with additional oxygen if cost-effective.

Aeration is achieved by injecting compressed air at the base of the fermenter through strategically placed orifices, typically near the stirring blades.

Microorganisms consume the dissolved oxygen in the medium and release carbon dioxide, which exists as carbonate and bicarbonate in equilibrium with the gas phase.



Systèmes d'aération et de stérilisation de l'air dans un bioréacteur de laboratoire.

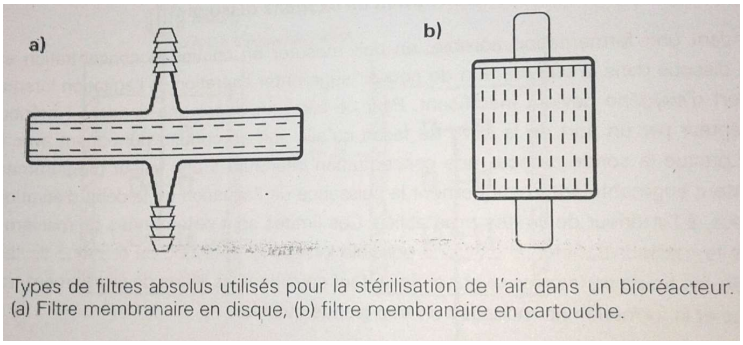
3.2 Sterilization of Air and Exhaust Gases

To ensure the aseptic conditions of the culture, the air must be sterilized before entering the reactor using so-called 'absolute' bacteriological filters.

These filters are also placed on the gas exhaust line, after the condenser.

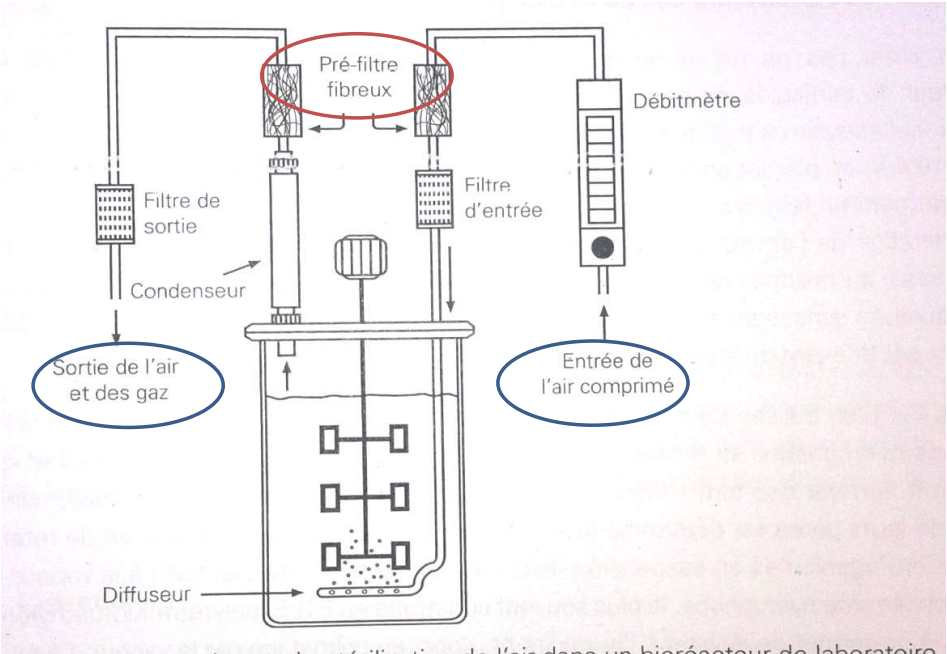
They are hydrophobic membrane filters (commonly made from PTFE – polytetrafluoroethylene) and are available in disk or cartridge form (pore sizes of 0.1, 0.20, or 0.45 µm).

These filters can be sterilized with steam and are resistant to fouling by water vapor



Types de filtres absolus utilisés pour la stérilisation de l'air dans un bioréacteur. (a) Filtre membranaire en disque, (b) filtre membranaire en cartouche.

Fibrous pre-filters are typically placed downstream of the absolute filters to minimize the risk of clogging them



Systèmes d'aération et de stérilisation de l'air dans un bioréacteur de laboratoire.

3.3 Measuring dissolved oxygen concentration

The dissolved oxygen concentration is continuously monitored during aerobic fermentations.

A probe inside the bioreactor remains in constant contact with the culture.

When the probe detects a concentration below a programmed threshold, a regulator automatically adjusts the agitation power, aeration flow rate, or both.

Oxygen sensors are typically membrane electrodes, which must be capable of being sterilized by steam under pressure.

There are two types of sensors:

Galvanic electrodes

Polarographic electrodes

4. Agitation

Agitation ensures homogenization and facilitates exchanges between the solid, liquid, and gas phases in the fermenter.

It also promotes thermal exchanges (heating or cooling) between the medium and the heat exchange system.

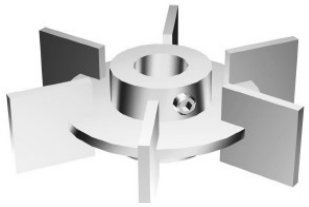
Agitation plays a crucial role in oxygen transfer during aerobic fermentations.

There are two types of stirring systems:

- ✓ Mechanical stirring system (rotary)
- ✓ Pneumatic stirring system (air-lift)

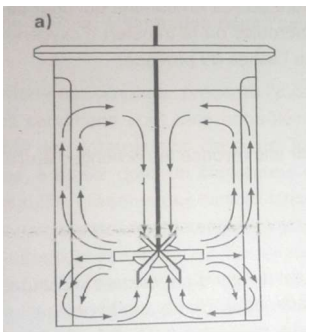
Mechanical stirring system (rotary)

Blade types



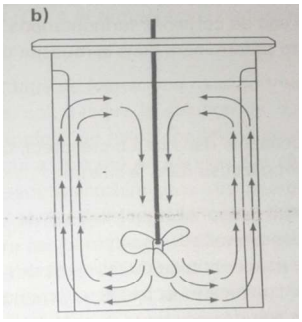
Rushton turbine

Marine turbine



Radial flow agitation

Easy oxygen transfer
Significant shear effect

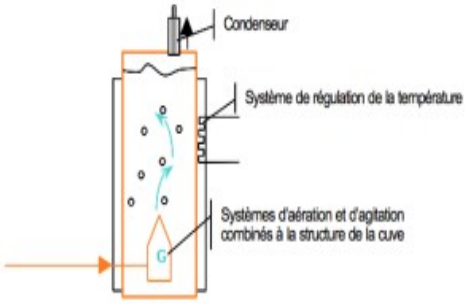


Axial flow agitation

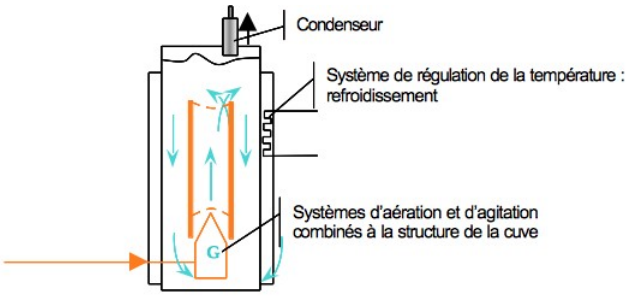
less oxygen transfer
Low shear effect

Pneumatic stirring system (air-lift)

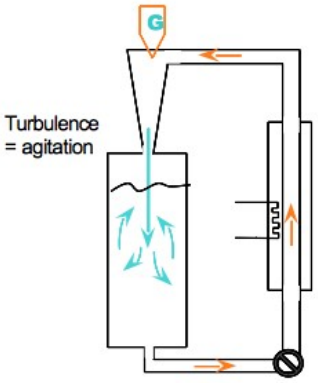
It involves blowing pressurized air from the bottom of a tall, narrow tank to create turbulence, which generates agitation in the bubble column



Bubble column



Internal loop ALB



jet fermenter (tubular loop)

Benefits

- ✓ Excellent oxygen transfer
- ✓ Minimal shear effect
- ✓ Suitable for cultures with high viscosity
- ✓ Suitable for cultures of molds or filamentous bacteria

5. Anti-foam control

In most aerobic fermentations, aeration and agitation result in the formation of foam on the surface of the culture, which can vary in density.

This foam is typically caused by organic polymers, such as proteins.

As the foam rises in the reactor, it can cause overflow, blocking the air filters, which significantly reduces aeration efficiency and increases the risk of contamination.

To mitigate this issue, the height-to-diameter ratio of the tanks can be increased, and they should only be partially filled (typically 2/3 to 3/4 of their total volume)

There are two methods for controlling foam:

1. Mechanical (foam breaker/disruptor): This method involves breaking the foam by rotating a turbine at high speed, mounted at the top of the axis. However, its efficiency is often low.

2. Automated addition of anti-foam agents: This method uses a probe and pump to automatically add anti-foam substances.

- The nature of the foam varies significantly depending on the culture and over time.
- It is crucial to select an appropriate anti-foam agent that provides both an immediate shock effect and a lasting impact, all while minimizing cost.
- The type of anti-foam used can also affect the extraction and purification of products

Some examples of antifoams

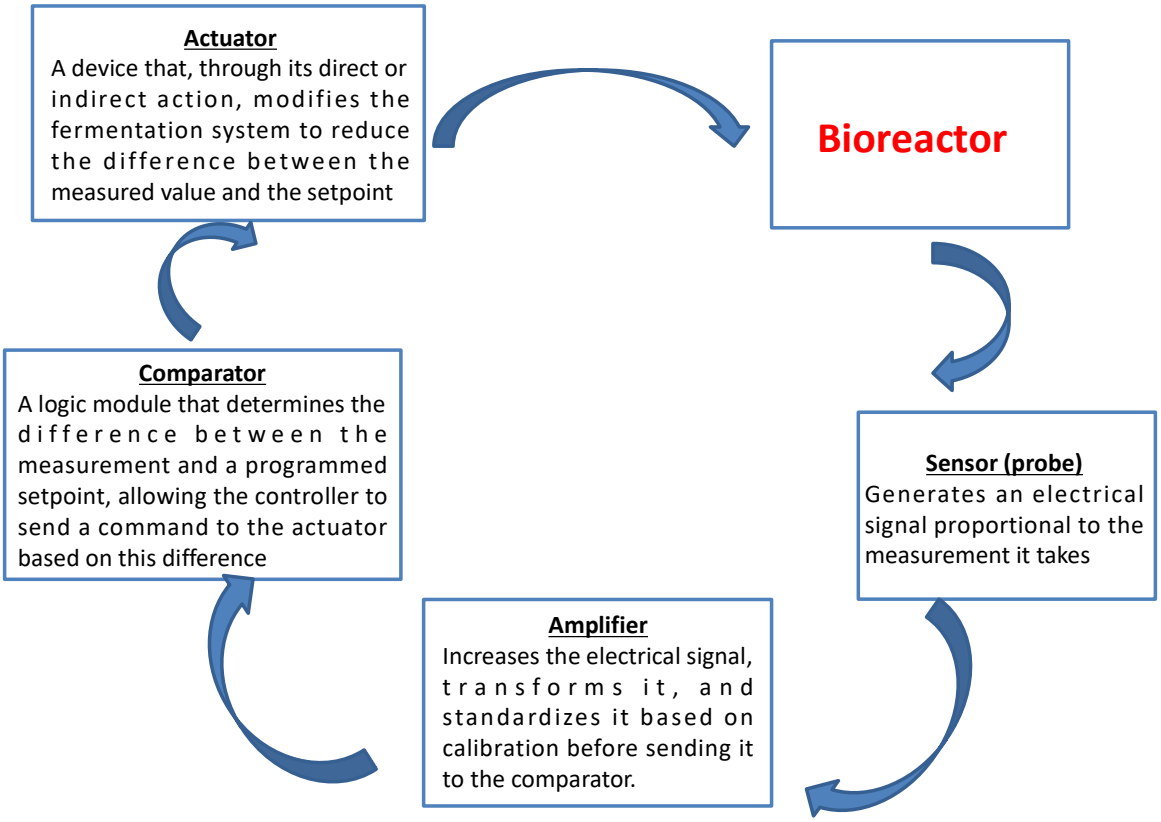
Table 9.1 Some antifoams which have been used in industry

Category	*Example*	*Chemical nature*	*Remarks*
Natrual oils and fats	Peanut oil, soybean oil	Esters of glycerol and long chain mono-basic acids	Not very efficient. Used as carriers for other antifoams; may be metabolized.
Alcohols	Sorbitan alcohol	Mainly alcohols with 8-12 carbon atoms	Not very efficient; may be toxic or may be metabolized.
Sorbitan derivatives	Sorbitan monolaureate (Span 20-Atlas)	Derivatives of sorbitol produced by reacting it with H_2SO_4 or ethylene	Span 20 active in extremely small amounts.
Polyethers	P400, P1200, P2000 (Dow Chemical Co.)	Polymers of ethylene oxide & propylene oxide	Active, but varies with fermentation.
Silicones	Antifoam A (Dow Corning Ltd.)	Polymers of polydimethyl -siloxane fluids	Very active; inert, highly dispersable, low toxity; expensive.

6. Regulation systems

Regulation involves automatically maintaining a minimal difference, ideally zero, between the actual value of a given fermentation parameter (e.g., temperature, pH, dissolved oxygen, etc.), measured by a probe, and the desired optimal value.

General diagram of a regulation loop



Types of Regulators

Depending on the type of action, regulators are classified as follows:

1. All-or-nothing action regulators: These regulators act only when there is a difference between the measurement and the setpoint, and their action is either fully on or fully off (e.g., cooling system).

2. Regulators with modulated on-off action: These regulators activate when there is a difference between the measurement and the setpoint, but their action is limited to a specific time period and is repeated if the difference persists (e.g., pH regulation system).

3. Proportional action regulators: The actuator sends a signal whose intensity is proportional to the difference between the measurement and the setpoint. As the difference decreases, the action becomes progressively weaker until equilibrium is reached, bringing the measurement closer to the setpoint (e.g., cascade control of dissolved oxygen concentration).

7. Medium and equipment sterilization

Traditional (protected) fermentations produce alcohols, organic acids, and antibiotics. These processes create favorable conditions by lowering pH and generating metabolites that inhibit the growth of foreign populations.

In modern fermentations, however, maintaining exemplary aseptic conditions is crucial to prevent microbial contamination. Such contamination can negatively impact the process's performance and yield.

As a result, rigorous sterilization of the medium, fermenter, and all associated equipment is mandatory to ensure the success of the fermentation process.

- Sterilization is the process of destroying or eliminating all microorganisms in a given environment, including vegetative cells, spores, and viruses.
- In fermentation, sterilization is essential to prevent contamination by foreign microorganisms that could compromise the process's performance.
- Contamination can significantly impact the yield and productivity of fermentation in several ways:

- Resource competition: It can consume part of the environment's resources for its own growth, depriving the producing microorganism.
- Product contamination: It may taint the finished product, rendering it unfit for consumption (e.g., microbial biomass contamination).
- Impurities: It can produce chemical compounds that complicate or prevent product purification.
- Product degradation: Through its metabolic activity, it can degrade the product (e.g., resistant bacteria contaminating antibiotic production).
- Host damage: It can threaten the survival of the producing microorganism (e.g., phage infections).

7.1. Media sterilization

The most commonly used sterilization methods include:

Thermal sterilization (moist heat): Moisture enhances heat transfer, making sterilization more efficient. When pressure increases, the sterilization temperature can exceed 100°C.

Microfiltration: A physical method that removes microorganisms by passing the medium through a fine filter.

I. Moist heat

Discontinuous

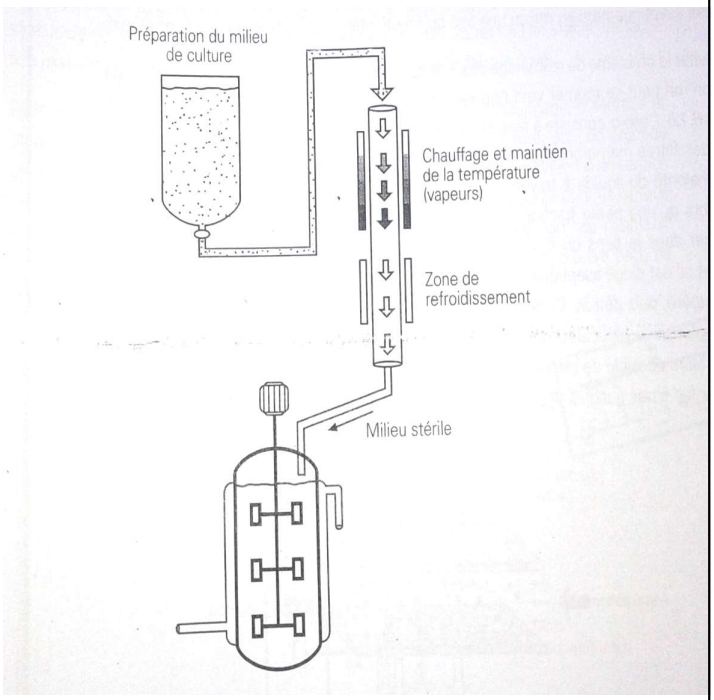
- Heat exchanger usage: A heat exchanger is employed to inject water vapor directly into the system (*in situ*).
- Agitation: Stirring enhances the speed of heat transfer, ensuring uniform temperature distribution.
- Heating and cooling cycle: The culture medium is heated to the operating temperature, maintained for the required duration, and then cooled. However, the heat treatment and cooling process can be relatively time-consuming, immobilizing the bioreactor.
- Steam injection: Sterilization can also be performed by injecting steam directly into the medium. This, however, dilutes the culture medium, which must be accounted for to avoid altering its composition (e.g., mineral balance).
- Separate sterilization: The medium can alternatively be sterilized in a separate tank, known as a "sterilization vessel." The sterilized medium is then transferred to the bioreactor under aseptic conditions to maintain sterility.

Continuous

For heat-sensitive culture media, continuous sterilization is performed using a heat exchanger (tubular or spiral).

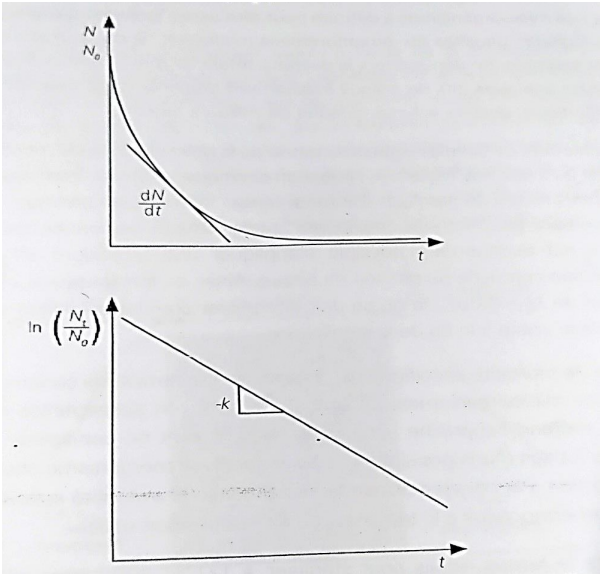
This method involves very high temperatures (140–150°C) applied for a short duration, typically just a few seconds.

This approach minimizes the destruction of growth factors and vitamins while maintaining sterilization efficiency.



Continuous sterilization of the culture medium through a system of heat exchangers

The kinetics of moist heat sterilization



Kinetics of microbial mortality during moist heat sterilization at a given temperature and pressure.
(a) Number of viable cells in sterilization time.
(b) Natural logarithm of the proportion of survivors in sterilization time.

$$N_t = N_0 e^{-kt} \quad \text{soit} \quad N_t/N_0 = e^{-kt}$$

N_0: number of viable cells at the start of the sterilization procedure
N_t: number of viable cells after a sterilization time t
t: sterilization time in minutes
k: specific mortality rate (min^{-1})

$$\ln(N_t/N_0) = -kt$$

So the required *sterilization time* will be calculated as follows:

$$t = [\ln (N_t/N_0)] / -k$$

Remarks:

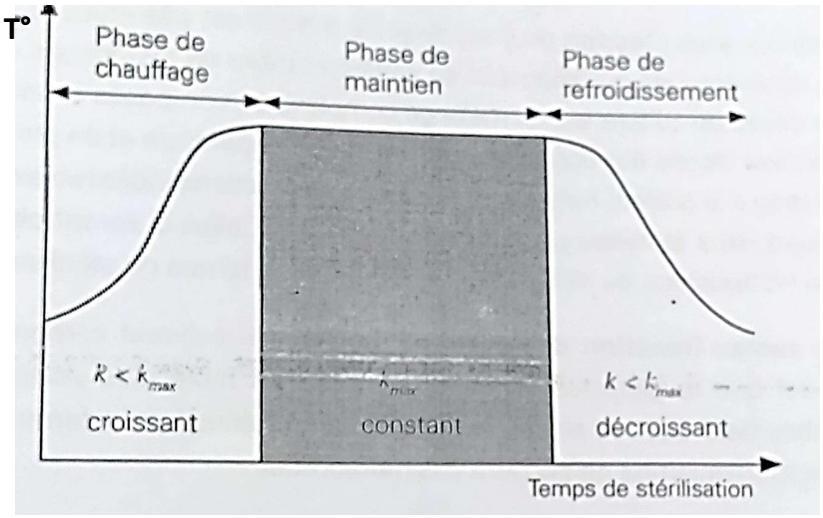
- Contaminants form a heterogeneous population of diverse microorganisms. To ensure maximum safety, we assume that the population is homogeneous and consists of microorganisms highly resistant to moist heat.
- By convention, the specific mortality rate (k) of *Bacillus stearothermophilus* spores at the sterilization temperature is used for this calculation.
- For Nt, we typically set a value of 0.001, which represents a 1 in 1000 risk that a viable cell will remain after the sterilization process.

Example:
To determine the time required to sterilize 1000 liters of a culture medium at 121°C, where the medium initially contains approximately 10^4 viable cells per milliliter, we calculate the sterilization time based on these conditions:
N_0 = 1000L x 10^4 cellules/ml x 1000 ml/L = 10^{10} cellules
N_t = 0,001 cellule
For *B. stearothermophilus*, k=2,54 min^{-1} à 121°C.
So:

$$t = [\ln (0{,}001/10^{10})] / -2{,}54 = 11{,}8 \text{ min}$$

Remarks

- For large volumes of culture media, which heat and cool gradually, sterilization is typically divided into three successive phases: heating, holding, and cooling.
- Cumulative mortality during the heating and cooling phases can reduce the required duration of the holding phase.



Variation in the T° of a medium during moist heat sterilization

Alteration of culture media by moist heat

- Caramelization of sugars (Maillard reaction)
- Degradation of thermolabile compounds
- pH variation
- Precipitation of certain inorganic salts
- Polymerization of certain organic compounds

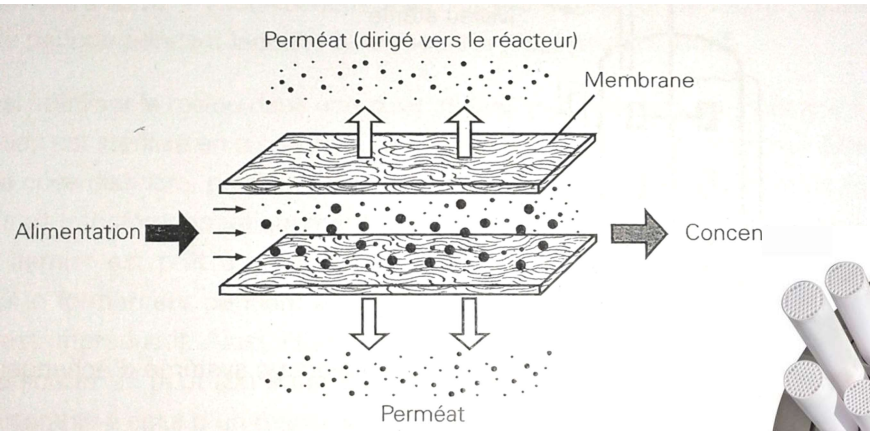
II. Microfiltration

- Conventional microfiltration (MF) involves forcing liquid through a polymer membrane with a specified pore diameter (e.g., 0.1 µm, 0.2 µm, or 0.45 µm). The membrane, sterilized by steam, is positioned perpendicular to the liquid's flow path.
- This filtration process does not eliminate viral particles or bacteriophages, which can be harmful to cell and microbial cultures.
- On a laboratory scale, it is suitable for preparing complete media.
- On an industrial scale, it is typically used for temperature-sensitive components, such as vitamins, antibiotics, and enzymes.

Bottle top filter used for the sterilisation of buffers

Example of Midicap filters being used in production

- The sterilization of large volumes of culture medium is performed using tangential microfiltration.
- This process involves circulating the liquid under pressure through a confined space between absolute ceramic membrane filters.

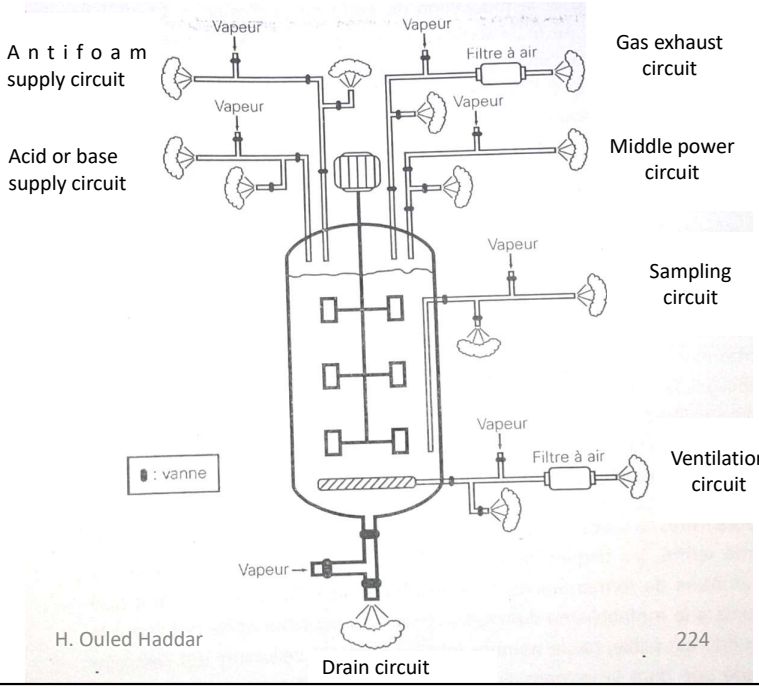


Représentation schématique de la filtration tangentielle.

H. Ouled Haddar

- The fermenter should be sterilized separately before introducing the sterile medium, as in the case of continuous sterilization tanks and tangential microfiltration systems.
- Steam is injected into the heat exchanger and the empty tank at 15 psi for 20 minutes to achieve sterilization.
- Devices and pipelines are sterilized individually using a valve system.

Sterilization of devices on a bioreactor



H. Ouled Haddar 224

Fermentation modes

I. Discontinuous culture process (batch)

This process is conducted in a closed environment, with no additions or sampling of the fermentation medium after inoculation, except for monitoring purposes.
The same medium is utilized throughout the growth, production, and accumulation phases.

Key characteristics:

- Low productivity
- Unproductive bioreactor for extended periods due to draining, cleaning, and preparation steps
- Minimal risk of contamination
- Minimal risk of mutations

Applications:

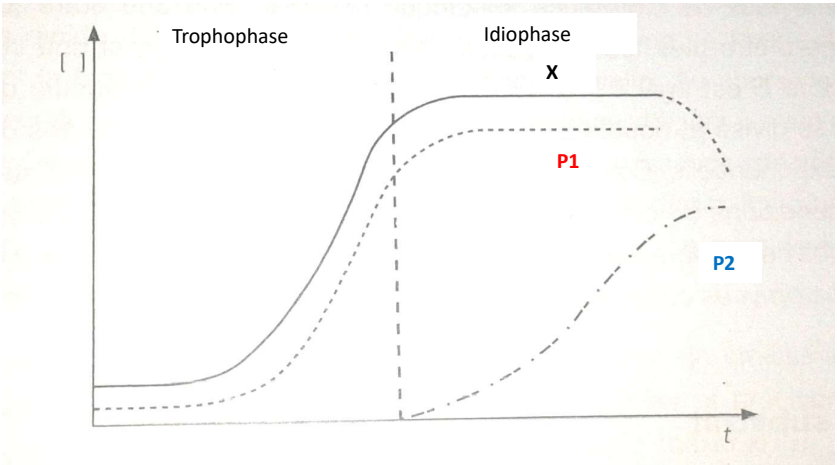
- Citric acid: Used as an acidulant in beverages, jams, confectionery, and other products. Produced by *Aspergillus niger* or *Candida guilliermondii*.
- Lactic ferments:

Streptococcus thermophilus and *Lactobacillus bulgaricus*: Used in yogurt production.
Streptococcus cremoris and *Penicillium roqueforti*: Used in cheese production.

- Antibiotics: Tetracycline Produced by *Streptomyces* species, Cephalosporins by *Acremonium*

- These fermentations are the oldest and most widely used on an industrial scale. The goal is to achieve the highest possible concentration of products or biomass (high productivity) in the shortest possible fermenter occupation time.

- Biomass increases according to the growth curve.
- The substrate decreases.
- The product increases.



Comparison of the production of a primary metabolite (P1) and a secondary metabolite (P2) in relation to growth

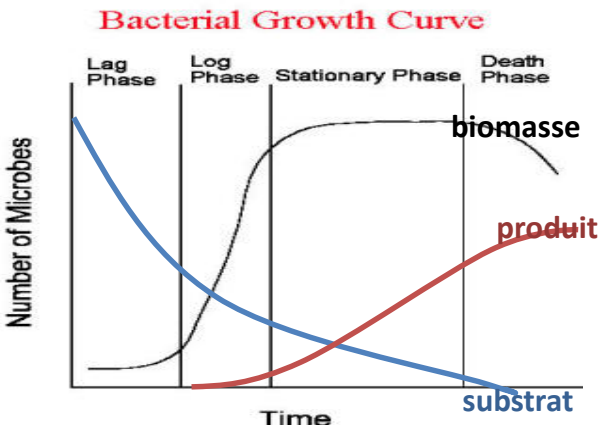
Kinetic
(perfectly mixed reactor)

Biomass balance (assessment)
(exponential phase)

$$dx/dt = \mu x$$

$$x_t = x_0 e^{\mu t}$$

$$\ln x_t = \ln x_0 + \mu t.$$



To calculate the generation time therefore t=G et $x_t=2x_0$

$$G = \ln 2 / \mu_{max}$$

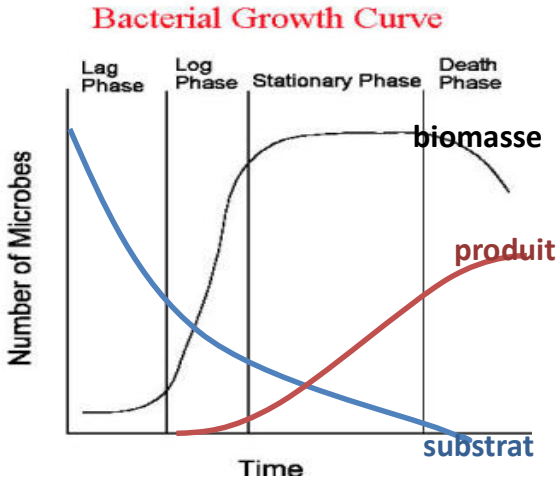
x: biomass concentration
x_0: initial biomass concentration
x_t: concentration of biomass at time t
μ: maximum specific growth rate (taux) (h^{-1})
t: time (hour)

Substrate utilization
(exponential phase)

$$V'''s = -dS/dt$$

$$[S]=([S_0]-[X_0].e^{\mu_{max}.t} - 1)/Y_{X/S}$$

$$Y_{X/S}=([X]-[X_0])/([S_0]-[S])$$



V'''s : volumetric rate of substrate utilization
x: biomass concentration
x_0: initial biomass concentration
S: substrate concentration
μ: maximum specific growth rate (taux) (h^{-1})
t: time (hour)
Yx/s: the biomass yield of the substrate

Substrate balance (stationary phase)

$$\mu = \mu_{max} s/(K_s + s)$$

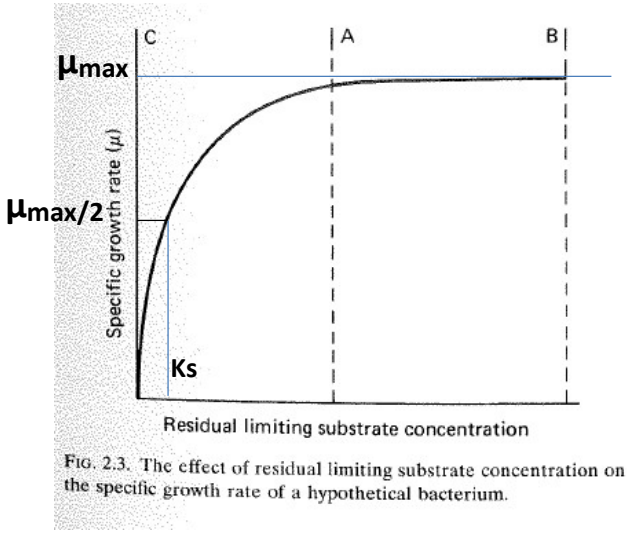


FIG. 2.3. The effect of residual limiting substrate concentration on the specific growth rate of a hypothetical bacterium.

μ_{max}: maximum specific growth rate (taux) (h^{-1})
S: substrate concentration
Ks: Monod's constant

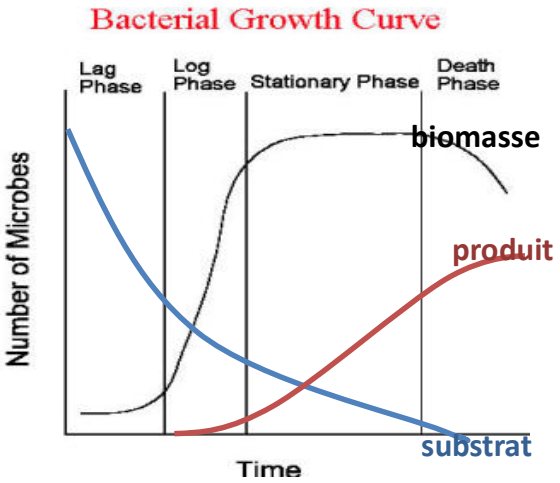
Product balance (assessment) (exponential phase)

$$V'''p = dP/dt$$

$$[P] = Y_{P/X} .[X_0].e^{\mu_{max}.t} - 1)$$

$$Y_{P/X} = ([P]-[P_0])/([X]-[X_0])$$

$$Y_{P/S} = ([P]-[P_0])/([S_0]-[S])$$



The evolution of concentrations can be more complex in many cases (non-constant yields, significant latency periods, sequential use of substrates, inhibition by the formed products , etc.)

V'''p : volumetric rate of the product appearance
P: product concentration
P_0: initial concentration of the product
μ: maximum specific growth rate (taux) (h^{-1})
t: time (hour)
$Y_{P/X}$: biomass product yield
$Y_{P/S}$: the product yield of the substrate

Productivity

Productivity is a variable that expresses the growth or production of a culture over time, typically measured in g/L/h

Biomass productivity (P_x)

$$P_x = (X_t - X_0)/(t - t_0)$$

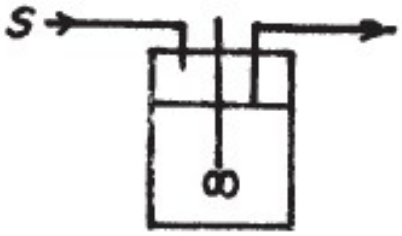
Product productivity (P_p)

$$P_p = (P_t - P_0)/(t - t_0)$$

II. Continuous cultivation process

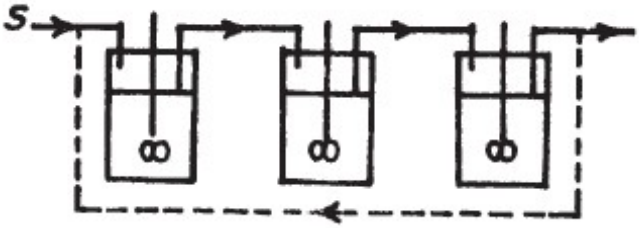
Continuous fermentation involves maintaining a balance of concentrations in the culture by continuously supplying the bioreactor with fresh medium, while simultaneously removing an equivalent volume of spent medium. This mode is primarily designed for growth-dependent products.

✓Chemostat
✓Turbidostat



✓Piston (tubular) reactor

✓Multi-stage reactor



- **Chemostat**

A chemostat is a type of continuous culture system where the growth rate of microorganisms is controlled by the rate of nutrient supply. The fresh medium is added at a constant rate, and an equivalent volume of culture is removed to maintain a constant culture volume. The concentration of a limiting nutrient (e.g., carbon or nitrogen) is kept constant, thus controlling the growth rate of the organisms.

- Key feature: Growth rate is limited by the rate of nutrient supply.

- **Turbidostat**

A turbidostat is a continuous culture system where the growth rate is controlled by the turbidity (cloudiness) of the culture, which correlates with cell concentration. The system adjusts the flow rate of the medium to maintain a constant optical density (OD) or cell density in the culture. The turbidity is continuously monitored, and the medium is added or removed to keep the culture at a steady cell concentration.

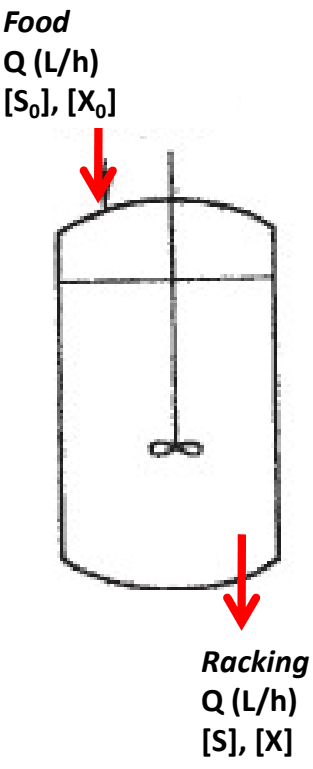
- Key feature: Growth rate is controlled by maintaining a constant cell density (turbidity).

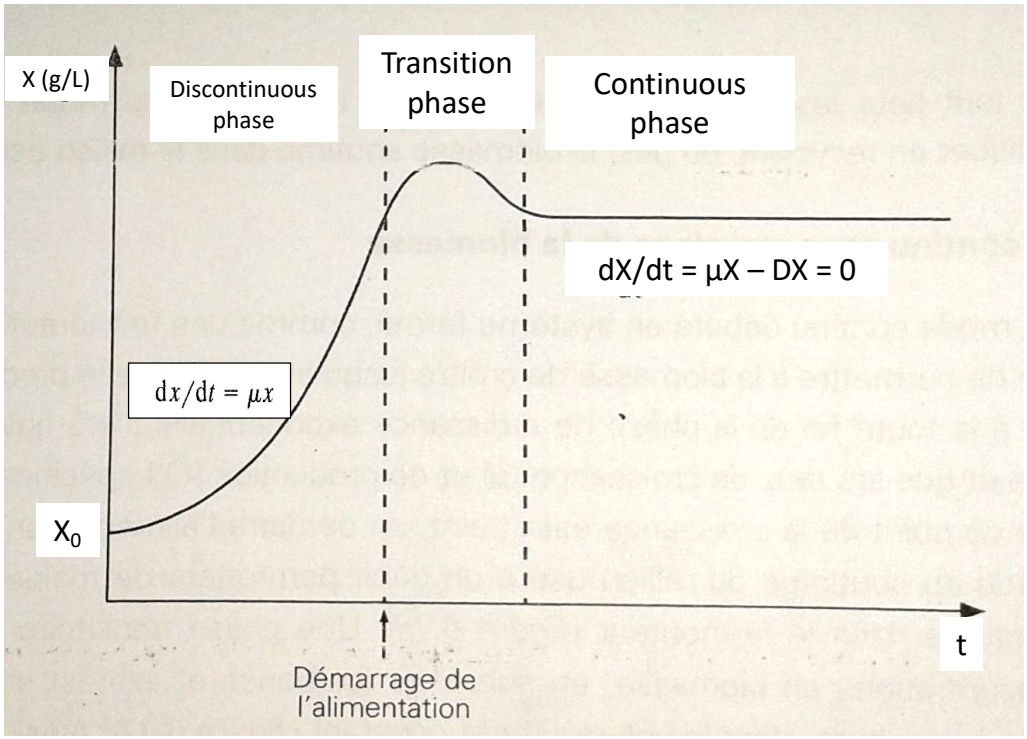
1. Continuous Mode Without Biomass Recycling

Continuous fermentation starts in a closed system, allowing the biomass to grow until it reaches the point of maximum production.

- The specific growth rate (μ) is at its maximum.
- The specific production rate is at its maximum.
- Biomass concentration is very high.

Once this optimal growth point is reached, the supply of fresh medium is initiated, and spent medium is removed at a flow rate (Q) that maintains constant concentrations in the system.





Growth kinetics of continuous fermentation

To establish continuous fermentation, it is necessary to define the flow rate (Q) of the medium entering and exiting the reactor to maintain constant concentrations in the culture.

Low flow rate: The substrate (S) is quickly consumed, leading to a drop in its concentration. Biomass (X) increases, but the substrate becomes limiting, causing a reduction in the specific growth rate (μ) and overall productivity.

High flow rate: Biomass is washed out of the culture, resulting in increased substrate concentration (S) and decreased biomass (X) and product concentration (P).

A continuous culture operates as an equilibrium system at constant volume, with perpetual dilution at a fixed rate.; so :

$$D = Q/V$$

D: dilution rate (h^{-1})
V : culture volume (L)
Q : Rate of medium flow (L/h)

Exemple:
V=2L, Q=0.5 L/h so D= 0.25 h^{-1}

(A quarter of the reactor's volume is renewed every hour, meaning the entire volume is replaced in 4 hours)

Biomass balance (ideal flow)

The variation in biomass concentration within the culture is determined by the difference between the growth rate and the biomass removal rate., i.e.:

$$dX/dt = \mu X - DX$$

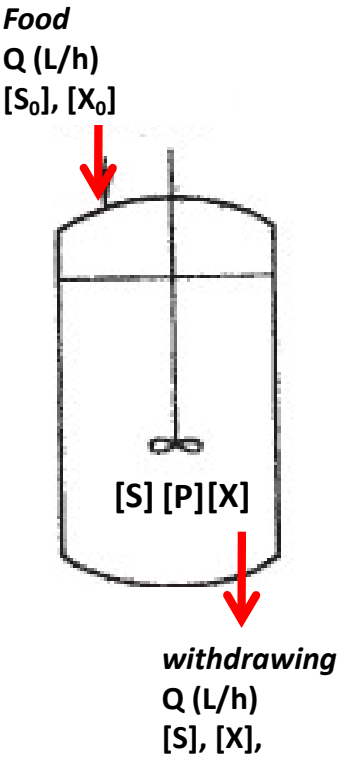
μX: biomass growth rate (g/L/h)
DX: biomass removal rate (g/L/h)

In balance:

$$dX/dt = \mu X - DX = 0$$

$$\mu X = DX$$

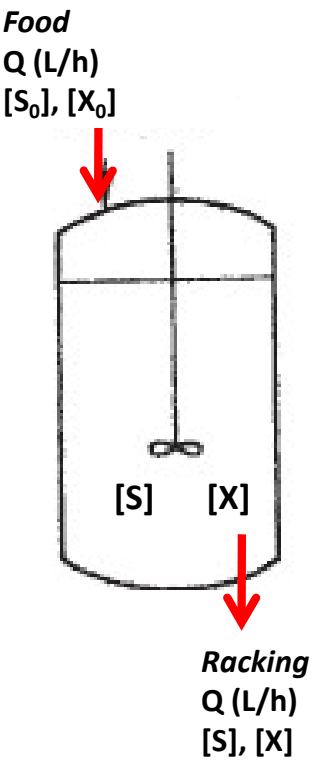
$$\boxed{\mu = D}$$



Substrate balance (steady state)

$$\boxed{[X] = Y_{X/S} \cdot ([S_0]-[S])}$$

S_0: concentration of added substrate
S: concentration of the substrate inside the reactor
μ: specific growth rate
Yx/s: the biomass yield of the substrate



Product review (steady state)

$$Y_{p/x} \cdot [X] = [P]$$

P: product concentration
$Y_{P/X}$: biomass product yield

Productivity

The biomass productivity (Px) of continuous mode fermentation is

$$P_x = DX = \mu X$$

P_x= biomass productivity (g/L/h)
X = biomass concentration (g/L)
D = dilution rate (h^{-1})
μ = specific growth rate (h^{-1})

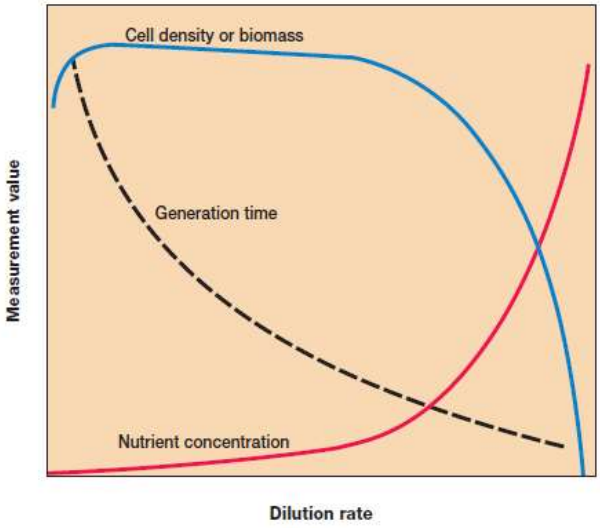


Figure 6.10 Chemostat Dilution Rate and Microbial Growth. The effects of changing the dilution rate in a chemostat.

Productivity

The product productivity (Pp) of a continuous fermentation is

$$P_p = dP/dt = H_pX$$

P_p= product productivity (g/L/h)
P= product concentration (g/L)
H_p= specific production rate (h^{-1})

$$H_p = \mu \, Y_{P/X}$$

Remarks

✓ In continuous fermentation, the actual product concentration in the culture withdrawn from the reactor is regularly measured and compared to the expected (calculated) value.

✓Over time, as cells divide at their maximum specific growth rate (μmax), mutations can arise in the system.

✓Non-productive mutant cells tend to accumulate, causing a gradual decline in productivity.

✓When this decline makes the process impractical, the reaction is typically stopped. As a result, continuous fermentation processes are usually limited to a few weeks.

Dilution Rate Control

To regulate the flow rate (Q) and dilution rate (D) during continuous fermentation, either a chemostat or a turbidostat is employed.

Chemostat

In a chemostat, the culture medium contains an essential nutrient (e.g., a carbon or nitrogen source) at a limiting concentration. This ensures that the specific growth rate (μ) remains below its maximum value, as described by the Monod relationship ($\mu < \mu_{max}$).

The concentration of the limiting substrate (S) is kept below the critical concentration (S') by adjusting the flow rate (Q) so that the dilution rate (D) remains slightly lower than its maximum permissible value ($D < \mu_{max}$).

Turbidostat

This bioreactor features a regulation system that controls the culture's absorbance by adjusting the flow rate (Q) and, consequently, the dilution rate (D).

An integrated photometer is connected to the supply and withdrawal pumps, enabling the maintenance of a constant biomass concentration (X).

This setup allows the system to maintain the dilution rate (D) at its maximum value, μ_{max}, without the risk of biomass washout.

Note
In practice, the dilution rate and biomass concentration are not perfectly constant. Instead, they oscillate within the limits of the regulation system's control.

Advantages and limitations of continuous fermentation

Benefits

- High productivity
- Minimized fermenter downtime
- Reduced material and labor costs (e.g., for emptying, cleaning, and refilling)

Limitations

- Elevated risk of microbial contamination
- Less suitable for producing growth-independent products (e.g., secondary metabolites)
- Requires the use of genetically stable strains
- High operational costs

1. Continuous mode with biomass recycling

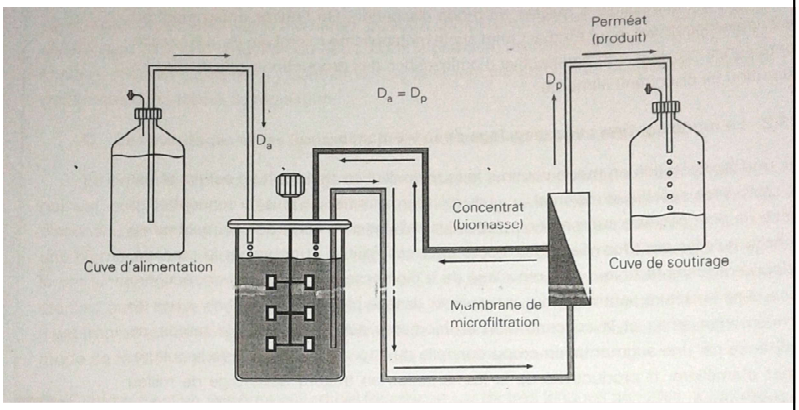
In continuous fermentation systems, the culture dilution rate is constrained by the maximum specific growth rate (µmax).

Additionally, the biomass concentration cannot be increased because cells are removed from the reactor at the same rate they are generated.

To address this limitation and enhance process efficiency, biomass recycling systems, such as membrane bioreactors, have been developed.

The culture withdrawn from the reactor is filtered under pressure in a tangential flow setup, using a membrane with absolute porosity.

This process separates the permeate, which contains the desired product, from the concentrate, which consists of biomass.



Fermentation in continuous mode with recycling of biomass by membrane microfiltration (membrane bioreactor) Da: Fresh medium feed rate, Dp: permeation rate

This technology shares the same limitations as conventional continuous fermentation. Additionally:

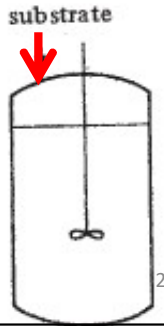
- The extremely high biomass concentrations in the reactor can clog the membranes, leading to premature termination of fermentation.

- At very high dilution rates, microorganisms may be unable to fully utilize the substrate, as it passes through the bioreactor too quickly. This unconsumed substrate ends up in the permeate, resulting in reduced product yield.

III. Fed batch culture process (fed-batch)

In this system, substrate (typically a carbon source) is added to a batch culture to regulate substrate concentration, growth, and production.

- Discontinuous mode with variable volume: Substrate is added directly to the tank, increasing the total volume.
- Discontinuous mode with fixed volume: Substrate is added without changing the overall volume of the culture.



Kinetics

- Growth Phase:

Initially, the system behaves like a traditional batch culture, with the cells growing as the available substrate is consumed.

- Substrate Limitation and Feeding:

When the substrate concentration becomes limiting (S decreases, causing μ to decrease), feeding begins with fresh medium at a constant flow rate.

The substrate concentration (S) is maintained constant and slightly below its initial value (S').

The growth rate (μ) stabilizes slightly below the maximum specific growth rate (μmax).

- Stationary Phase:

Once the tank is filled, the substrate feed is stopped. The culture then transitions into the stationary phase as the substrate concentration (S) decreases further, leading to a cessation of growth (μ = 0).

Advantages of this system are:

- Prevention of Substrate Inhibition:

Maintaining a low concentration of the limiting substrate helps avoid inhibition caused by excess substrate.

- Reduction of Respiratory Inhibition (Crabtree Effect):

By controlling substrate levels, the inhibitory effect on respiration (e.g., in *E. coli* and baker's yeast) is minimized.

- Optimized Secondary Metabolite Production:

The system maintains the specific growth rate below µmax, which is ideal for the production of secondary metabolites.

- Mitigation of Feedback Inhibition:

Excess product is diluted effectively, reducing feedback inhibition and maintaining productivity.