Bioreactors

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GLOSSARY

Austenitic stainless steels Stainless steels with an specific type of nonmagnetic crystal structure.

Downcomer The region of a an airlift bioreactor where the gas–liquid dispersion flows downwards.

Enzymes Protein molecules that catalyze the various biochemical reactions.

Heterotrophic growth Growth in which the carbon and energy for building the biomass are derived exclusively from organic chemicals.

Mass transfer Molecular-level transport of any substance through any medium.

Organelles Well-defined structures associated with specific functions in a cell.

Photomixotrophic culture A culture that obtains a part of the carbon needed for making the biomass from an organic chemical and another part from carbon dioxide via photosynthesis

Phototrophic growth Growth in which the carbon needed to make the biomass comes from fixation of carbon dioxide via photosynthesis.

Pressure drop Loss of pressure with distance downstream from any point in tubes, channels, and other flow devices.

Product (or substrate) inhibition A situation in which the increasing concentration of the product (or substrate) of a reaction slows down the rate of the reaction by interfering with the enzyme(s) that catalyze the reaction.

Protoplast A cell with its wall removed.

Reduced substrate A substrate that contains relatively little oxygen within its molecules.

Riser The region of an airlift bioreactor where the gas-liquid dispersion flows upwards.

Solidity ratio The ratio of the swept area to the total projected area of the impeller blades, as viewed from directly overhead an installed impeller.

Substrate Any compound that is modified by a cell or an enzyme.

Water-for-injection (WFI) Highly purified water that conforms to the WFI specifications of the United States Pharmacopoeia.

A BIOREACTOR is any device or vessel that is used to carry out one or more biochemical reactions to convert any starting material (or raw material or *substrate*) into some product. The conversion occurs through the action of a *biocatalyst*—enzymes, microorganisms, cells of animals and plants, or subcellular structures such as chloroplasts and mitochondria. The starting substrate may be a simple

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organic chemical (e.g., sugar and penicillin), an inorganic chemical such as carbon dioxide, or a poorly defined complex material such as meat and animal manure. The product of the conversion may be cells (or biomass), viruses, and chemicals of various kinds. The processes occurring in a bioreactor may be summarized as follows:

$$\text{Substrates} \xrightarrow{\text{biocatalyst(s)}} \text{products.}$$

Many different kinds of bioreactors are available and sometimes a given type may be operated in different ways to obtain different results. The different bioreactor designs are needed to accommodate the great diversity of substrates, products, and biocatalysts and the different requirements of the different bioconversion processes.

I. INTRODUCTION

Bioreactors are used in all kinds of bioprocesses, including those for making foods such as soy sauce; those for treating domestic and industrial wastewater; and ones for making vaccines, antibiotics, and many other useful chemicals. Bioreactors that produce microbial cells and cells of animals and plants, are known as *fermenters*. In addition to cells, a fermenter may also produce other chemicals, or convert (or biotransform) a chemical added to the fermenter, to a different molecule. A fermenter may contain either a single cell type (i.e., *monoseptic* operation), or a mixed population of different kinds of cells. Fermenters that operate monseptically are designed as sealed units, with barriers that prevent ingress of contaminating microorganisms from the environment. Other types of bioreactors may contain only nonviable entities (i.e., ones that cannot multiply) including cells, isolated enzymes, and organelles obtained from cells. The cells and organelles may be freely suspended in an aqueous medium, or they may be confined by various methods of immobilization. Unlike cells and organelles, enzymes are usually soluble in aqueous media; for repeated use, a soluble enzyme may be retained in the bioreactor by ultrafiltration membranes, or the enzyme may be immobilized in an insoluble matrix.

II. BIOREACTOR SYSTEMS

In most bioprocessing situations cells and biocatalysts are submerged and suspended in a broth that sustains live cultures and dissolves the chemicals that are being modified by the action of the biocatalyst. Bioreactors for submerged processing are generally quite different from those used in solid-state cultivation. Solid-state fermentations are carried out with a moistened solid substrate in the absence of free water, e.g., during composting, making of hard cheeses, and fermentation of cocoa beans for chocolate. Submerged processing is widely used in treatment of wastewater and production of vaccines, antibiotics, and many other useful products. Bioreactors for submerged and solid-state processes are discussed next.

A. Submerged Culture

1. Mechanically Stirred Tank Bioreactors

Stirred tank bioreactors consist of a cylindrical vessel with a motor driven central shaft that supports one or more agitators (Fig. 1). Different kinds of agitators are used in different applications. Microbial culture vessels are generally provided with four baffles placed equidistant around the periphery of the tank. The baffles project into the vessel from near the walls (Fig. 1). The baffles run the entire working height of the vessel and they prevent swirling and vortexing of the fluid. The baffle width is 1/10 or 1/12 of the tank diameter. A gap of about 1.5% of tank diameter is left between the wall and the baffle to prevent stagnation of fluid near the wall. The working aspect ratio of the vessel is between 3 and 5, except in animal cell culture applications where aspect ratios do not normally exceed 2. Often, the animal cell culture vessels are unbaffled.

The number of impellers used depends on the aspect ratio of the vessel. The lowermost impeller is located about one third of the tank diameter above the bottom of the tank. Additional impellers are spaced with

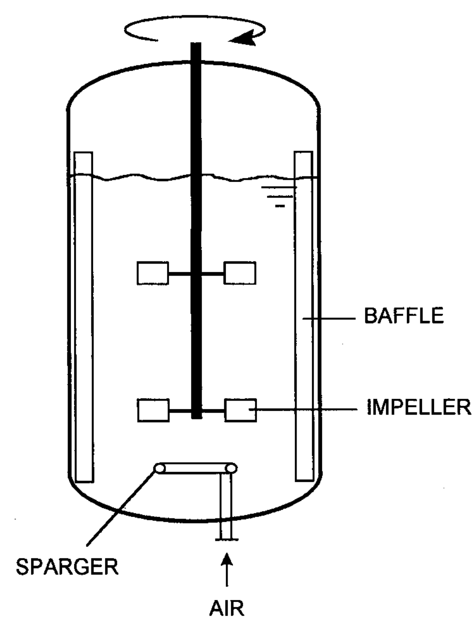


FIGURE 1 Mechanically stirred bioreactor.

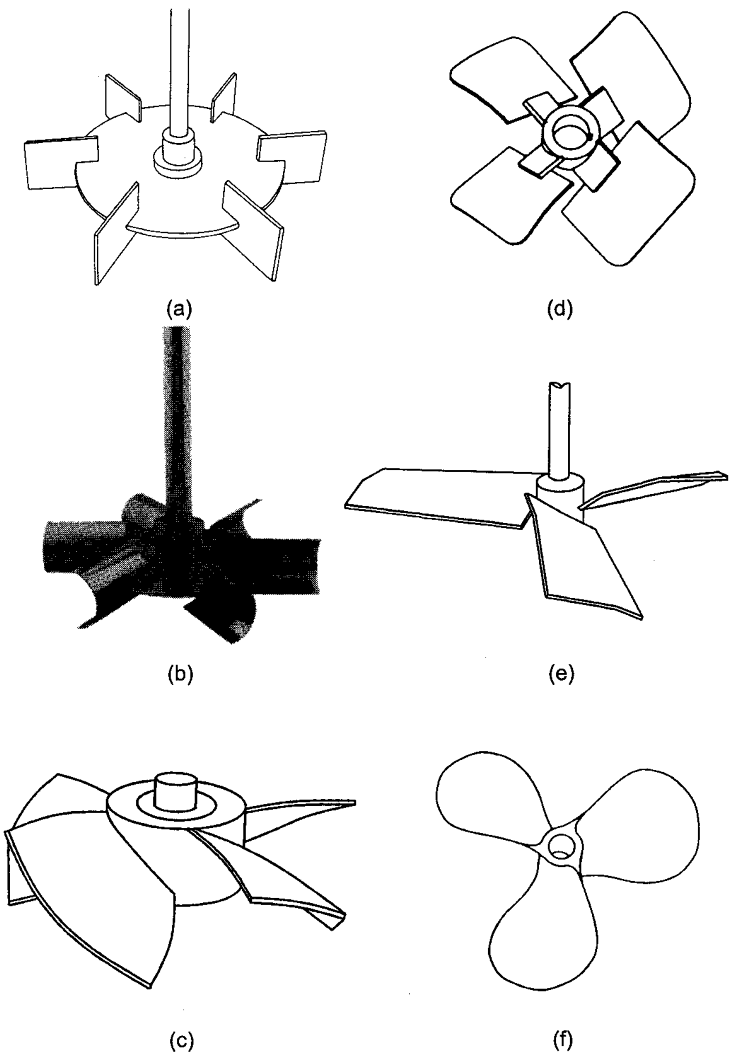


FIGURE 2 Agitators or impellers used in stirred bioreactors: (a) Rushton disc turbine; (b) concave bladed turbine; (c) Prochem Maxflo T hydrofoil; (d) Lightnin' A315 hydrofoil; (e) Chemineer hydrofoil; and (f) marine propeller.

1.2-impeller-diameter distance in between. The impeller diameter is about 1/3 of the vessel diameter for gas dispersion impellers such as Rushton disc turbines and concave bladed impellers (Fig. 2a, b). Larger hydrofoil impellers (Fig. 2c–e) with diameters of 0.5 to 0.6 times the tank diameter are especially effective bulk mixers and are used increasingly. High solidity ratio hydrofoils (Fig. 2c, d) are good for highly viscous mycelial broths. Animal cell culture vessels typically employ a single large-diameter, low-shear impeller such as a marine propeller (Fig. 2f). Oxygen is provided typically by sparging the broth with sterile air. In microbial fermenters, gas is sparged below the lowermost impeller using a perforated pipe ring sparger with a ring diameter that is slightly smaller than that of the impeller (Fig. 1). A single hole sparger discharging the gas below the impeller at the tank centerline is used sometimes. Aeration velocity is usually kept at less than $0.05\ \mathrm{ms^{-1}}$, or the mixing effectiveness of the impeller will be reduced. In bioreactors for animal cell culture, the aeration velocities are lower, usually less than $0.01\ \mathrm{m\ s^{-1}}$, and the gas is sparged such that it does not rise through the region swept by the impeller. Mixing, oxygen transfer, and heat transfer improve with increasing agitation and aeration rates. In low viscosity media, the type of impeller has little effect on the gas–liquid mass transfer rate, so long as the power input per unit liquid volume is kept unchanged.

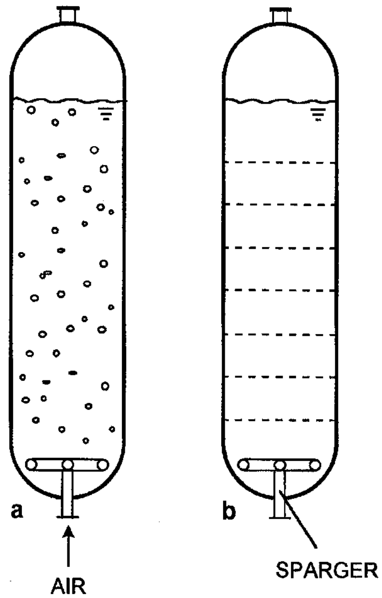


FIGURE 3 Bubble columns: (a) the basic design, (b) a column with transverse perforated baffle plates, (c) a column with vertical baffles, and (d) a column with corrugated sheet static mixers for gas dispersion.

2. Bubble Columns

A bubble column consists of a gas sparged pool of liquid or slurry (Fig. 3a). Usually, the column is cylindrical and the aspect ratio is between 4 and 6. This basic design may be modified by placing various kinds of internals—e.g., horizontal perforated plates, vertical baffles, and corrugated sheet packings—inside the vessel (Fig. 3b–d). Gas is sparged at the base of the column through perforated pipes, perforated plates, or sintered glass or metal microporous spargers. Oxygen transfer, mixing, and other performance factors are influenced mainly by the gas flow rate and the properties of the fluid. The column diameter does not affect its behavior so long as the diameter exceeds 0.1 m. One exception is the mixing performance. For a given gas flow rate, the mixing improves with increasing vessel diameter. Mass and heat transfer performance improves as gas flow rate is increased. In bubble columns the maximum aeration velocity usually remains less than 0.1 ms^{-1}. The liquid flow rate does not influence the gas–liquid mass transfer coefficient so long as the superficial liquid velocity remains below 0.1 ms^{-1}.

Bubble columns with recirculation and airlift bioreactors are especially suited to hairy root culture of plant cells. The rootlets tend to grow as an entangled static mass with a doubling time of about 2 days. The fluid flowing past the roots supplies oxygen and other nutrients. A bubble column bioreactor with the hairy root mass confined between two perforated retention plates, is shown in Fig. 4. The nutrient medium flowing into the column may be oxygenated in a separate column, or air may be bubbled in the column that contains the root mass. External oxygenation is suitable when the conditions (oxygen consumption, fluid residence time) in the column with the root mass are such that the spent medium leaving the column is not totally depleted of oxygen.

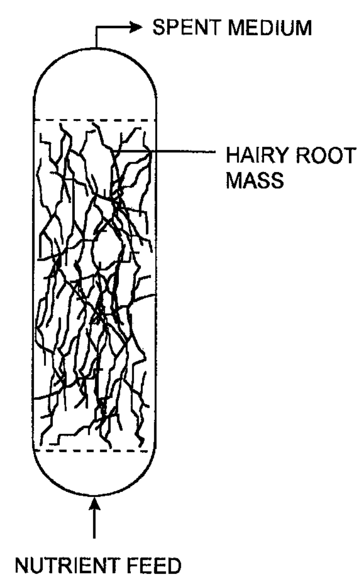


FIGURE 4 Bubble column bioreactor for hairy root cultivation.

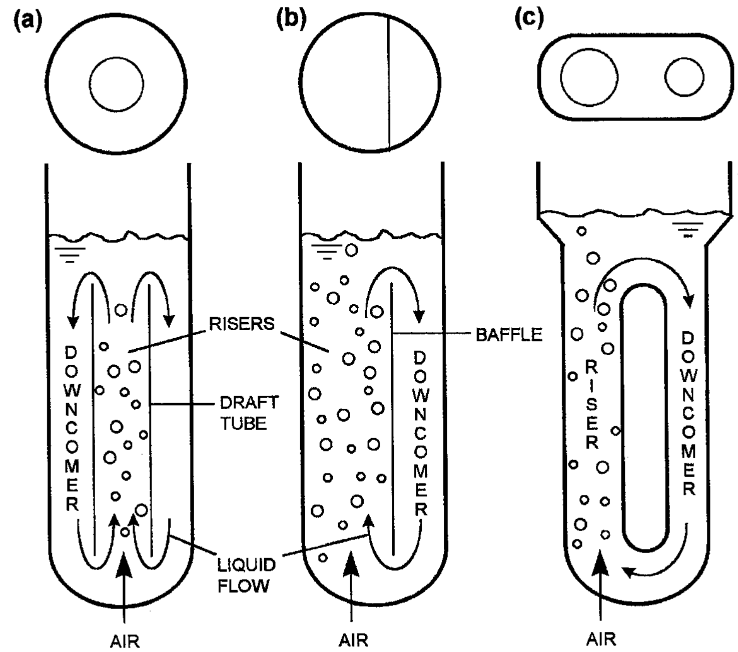


FIGURE 5 Airlift bioreactors: (a) draft-tube internal-loop configuration, (b) a split-cylinder device, and (c) an external-loop system.

3. Airlift Bioreactors

In airlift bioreactors the fluid volume of the vessel is divided into two interconnected zones by means of a baffle or draft-tube (Fig. 5). Only one of these zones is sparged with air or other gas. The sparged zone is known as the riser; the zone that receives no gas is the downcomer (Fig. 5a–c). The bulk density of the gas-liquid dispersion in the gas-sparged riser tends to be less than the bulk density in the downcomer; consequently, the dispersion flows up in the riser zone and downflow occurs in the downcomer. Sometimes the riser and the downcomer are two separate vertical pipes that are interconnected at the top and the bottom to form an external circulation loop (Fig. 5c). External-loop airlift reactors are less common in commercial processes compared to the internal-loop designs (Fig. 5a, b). The internal-loop configuration may be either a concentric draft-tube device or an split-cylinder (Fig. 5a, b). Airlift reactors have been successfully employed in nearly every kind of bioprocess—bacterial and yeast culture, fermentations of mycelial fungi, animal and plant cell culture, immobilized enzyme and cell biocatalysis, culture of microalgae, and wastewater treatment.

Airlift bioreactors are highly energy efficient relative to stirred fermenters, yet the productivities of both types are comparable. Heat and mass transfer capabilities of airlift reactors are at least as good as those of other systems, and airlift reactors are more effective in suspending solids than are bubble columns. For optimal gas–liquid mass transfer performance, the riser-to-downcomer cross-sectional area ratio should be between 1.8 and 4.3 in an airlift reactor. All performance characteristics of airlift bioreactors are linked ultimately to the gas injection rate and the resulting rate of liquid circulation. The liquid circulation velocity depends on the difference in gas holdup (i.e., the volume fraction of gas in the gas-liquid dispersion) between the riser and the downcomer. Liquid velocity is affected also by the geometry of the reactor and the viscosity of the fluid. In general, the rate of liquid circulation increases with the square root of the height of the airlift device. Consequently, the reactors are designed with high aspect ratios of at least 6 or 7, or even in the hundreds. Because circulation is driven by the gas holdup difference between the riser and the downcomer, circulation is enhanced if there is little or no gas in the downcomer. All the gas in the downcomer comes from being dragged in with the liquid as it flows into the downcomer from the riser near the top of the reactor (Fig. 5). Various designs of gas-liquid separators (Fig. 6) are sometimes used in the head zone to reduce or eliminate the gas carry over to the downcomer. Most gas–liquid separators work in one of two ways: either the horizontal flow path between the riser and the downcomer is extended (Fig. 6a) so that the liquid resides for a longer period in the head zone and this provides sufficient time for the gas bubbles to disengage; or the entrance region of the downcomer is expanded in cross section (Fig. 6b) so that the downward flow velocity of the liquid is reduced and it no longer drags gas bubbles into the downcomer. Relative

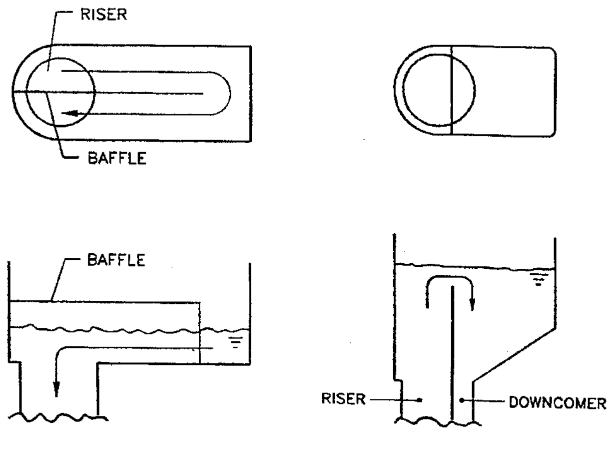


FIGURE 6 Gas–liquid separators for airlift bioreactors: (a) extended length of the flow path in the head zone, (b) enlarged entrance cross section of the downcomer zone.

to a reactor without a gas–liquid separator, installation of a suitably designed separator will always enhance liquid circulation, i.e., the increased driving force for circulation will more than compensate for any additional resistance to flow due to the separator.

4. Fluidized Beds

Fluidized bed bioreactors are suited to reactions involving a fluid-suspended particulate biocatalyst such as the immobilized enzyme and cell particles or microbial flocs. An up-flowing stream of liquid is used to suspend or "fluidize" the relatively dense solids (Fig. 7). Geometrically, the reactor is similar to a bubble column except that the cross section is expanded near the top to reduce the superficial velocity of the fluidizing liquid to a value below that needed to keep the solids in suspension (Fig. 7). Consequently, the solids sediment in the expanded zone and drop back into the narrower reactor column below; hence, the solids are retained in the reactor whereas the liquid flows out. A liquid fluidized bed may be sparged with air or some other gas to produce a gas–liquid–solid fluid bed. If the solid particles are too light, they may have to be artificially weighted, for example, by embedding stainless steel balls in an otherwise light solid matrix. A high density of solids improves solid–liquid mass transfer by increasing the relative velocity between the phases. Denser solids are also easier to sediment, but the density should not be too great relative to that of the liquid, or fluidization will be difficult.

Liquid fluidized beds tend to be fairly quiescent but introduction of a gas substantially enhances turbulence and agitation. Even with relatively light particles, the superficial liquid velocity needed to suspend the solids may be so high that the liquid leaves the reactor much too quickly, i.e., the solid–liquid contact time may be insufficient for the reaction and the liquid may have to be recycled to obtain a sufficiently long cumulative contact time with the biocatalyst. The minimum fluidization velocity—i.e., the superficial liquid velocity needed to just suspend the solids from a settled state—depends on several factors, including the density difference between the phases, the shape and diameter of the particles, and the viscosity of the liquid.

5. Packed Bed Bioreactors

A bed of solid particles usually with confining walls (Fig. 8) constitutes a packed bed. The biocatalyst is supported on or within the solid matrix that may be porous or a homogeneous non-porous gel. The solids may be ridged, or only slightly compressible. The particles may be randomly shaped (e.g., wood chips and rocks) or they may be uniform spheres, cylinders, cubes, or some other shape. A fluid containing dissolved nutrients and substrates flows through the solid bed to provide the needs of the immobilized biocatalyst. Metabolites and products are released into the fluid and are taken out with the flow. The flow may be upward or downward, but downflow under gravity (i.e., trickle bed operation) is the norm specially if the immobilized biocatalyst requires oxygen (Fig. 8). If the fluid flows up the bed, the maximum flow velocity is limited because the velocity cannot exceed the minimum

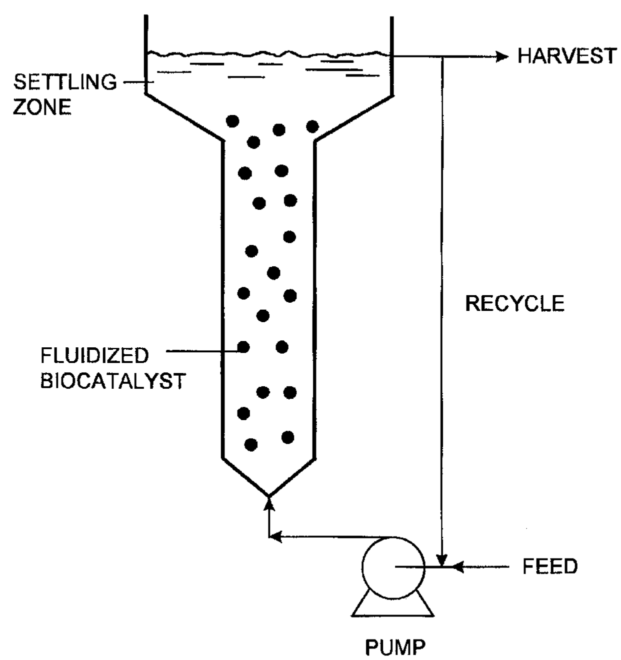


FIGURE 7 A fluidized bed bioreactor with recycle of medium.

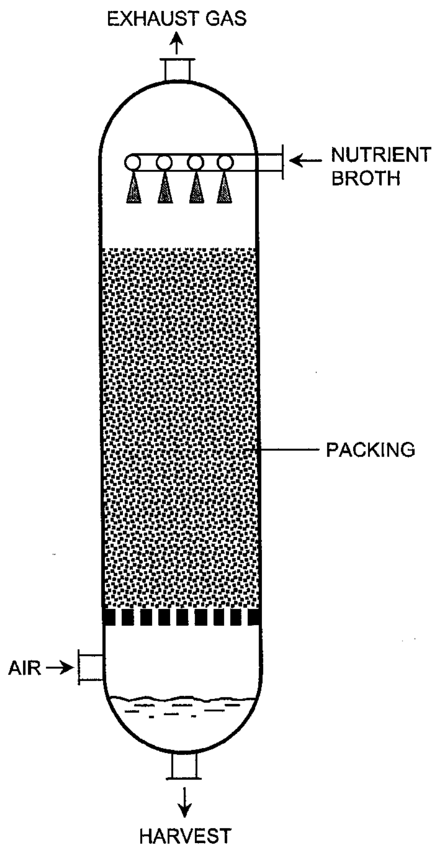


FIGURE 8 A packed bed bioreactor.

fluidization velocity or the bed will fluidize. The depth of the bed is limited by several factors, including the density and the compressibility of the solids, the need to maintain a certain minimal level of a critical nutrient such as oxygen through the entire depth, and considerations of the maximum acceptable pressure drop. For a given voidage—or solids-free volume fraction of the bed—the gravity driven flow rate through the bed declines if the depth of the bed is increased. Nutrients and substrates are depleted as the fluid moves down the bed. Conversely, concentrations of metabolites and products increase. Thus, the environment of a packed bed is nonhomogeneous, but concentration variations along the depth can be reduced by increasing the flow rate. Gradients of pH may occur if the reaction consumes or produces the H^+ ion. Because of poor mixing, pH control by addition of acid and alkali is nearly impossible. Beds with greater voidage permit greater flow velocities through them, but the concentration of the biocatalyst in a given bed volume declines as the voidage is increased. If the packing—i.e., the biocatalyst-supporting solids—is compressible, its weight may compresses the bed unless the packing height is kept low. Flow is difficult through a compressed bed because of a reduced voidage. Packed beds are used especially commonly as immobilized enzyme reactors and "biofilters" for the treatment of gaseous pollutants. Such reactors are particularly attractive for product inhibited reactions: the product concentration varies from a low value at the inlet of the bed to a high value at the exit; thus, only a part of the biocatalyst is exposed to high inhibitory levels of the product. In contrast, if the catalyst particles were suspended in a well mixed stirred vessel, all the catalyst will experience the same inhibitory product concentration as in the fluid stream that leaves the reactor.

6. Photobioreactors

Photobioreactors are used for photosynthetic culture of cyanobacteria, microalgae, and to a much lesser extent, cells of macroalgae (seaweeds) and plants. Photosynthesis requires light and light stimulates some cultures in ways not seen in purely heterotrophic growth. Because of the need to provide light, photobioreactors must have a high surface-to-volume ratio and this greatly affects the design of bioreactor. The demand for light is reduced in photomixotrophic culture where an organic compound is the major source of carbon for the cells and only a limited amount of photosynthesis (i.e., the fixation of carbon dioxide in presence of light) takes place.

Only a few phototrophic microorganisms mainly cyanobacteria and microalgae are cultured on large scale. This kind of mass culture is carried out in photobioreactors open to atmosphere, e.g., in ponds, lagoons, and "raceway" channels (Fig. 9). The latter are widely used and

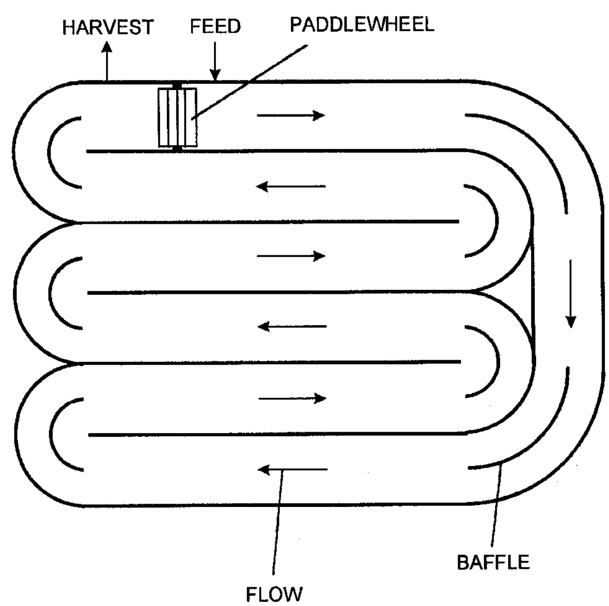


FIGURE 9 A closed-loop raceway channel for outdoor culture of photosynthetic microorganisms.

they consist of a closed loop recirculation channel that is about 0.4 m or less in depth. The culture is mixed and circulated by a paddle wheel (Fig. 9) or pumps. The channel is built in concrete and may be lined with plastic. The culture feeding and harvest are continuous, except during the night; however, the channel keeps circulating even during the night. Use of open photobioreactors is limited to only a few microbial species—the astaxanthin producer *Dunaliella*, *Chlorella*, and *Spirulina*. The last two are used mostly as healthfood. These few species can be grown in selective environments (e.g., highly alkaline and saline) that suppress contamination by other microorganisms.

A greater variety of algae and tissue cells may be cultured in fully closed photobioreactors; however, like the open raceways and ponds, a closed photobioreactor must have a high surface-to-volume ratio to effectively capture light and this requirement greatly increases the installation and operational expenses of these systems. Closed photobioreactors are used mainly to produce biomass for aquaculture feeds. A closed photobioreactor consists of a light capture unit or photoreceiver, a pumping device to circulate the culture through the photoreceiver, and a gas-exchange column to remove the photosynthetically generated oxygen and provide the carbon source (carbon dioxide). Most useful photoreceivers or photocollectors are made of ridged or flexible plastic tubing. Glass tubing is used in some cases.

The tubes may be arranged into a ladder configuration (Fig. 10a) that may be installed flat on the ground or it may be positioned at a 90° angle, as a fence. A continuous run tube may be formed into a serpentine configuration (Fig. 10b) or it may be made into a "biocoil." The latter is obtained by helically winding a flexible polymer tubing on a cylindrical-shaped frame made of aluminum or other metal bars (Fig. 10c). The culture tubes are 3 cm in diameter. The continuous run length of a single tube depends on the oxygen generation rate and the culture velocity. The length of a single tube is usually 50 m, or less. Several sections of continuous run tubing are installed on a given frame and common headers are used to distribute and collect the broth. The height of the coil may be up to 8 m and the diameter may be 2 m or more. (A larger diameter improves illumination in the region enclosed by the coil. The optimum diameter depends on the height of the coil.) The culture is circulated by a pump or an airlift device. The airlift column or a separate tower is used for gas-exchange. Temperature is controlled by evaporative cooling of water sprayed on the solar receiver. Also, a tubular heat exchanger may be used instead of evaporative cooling.

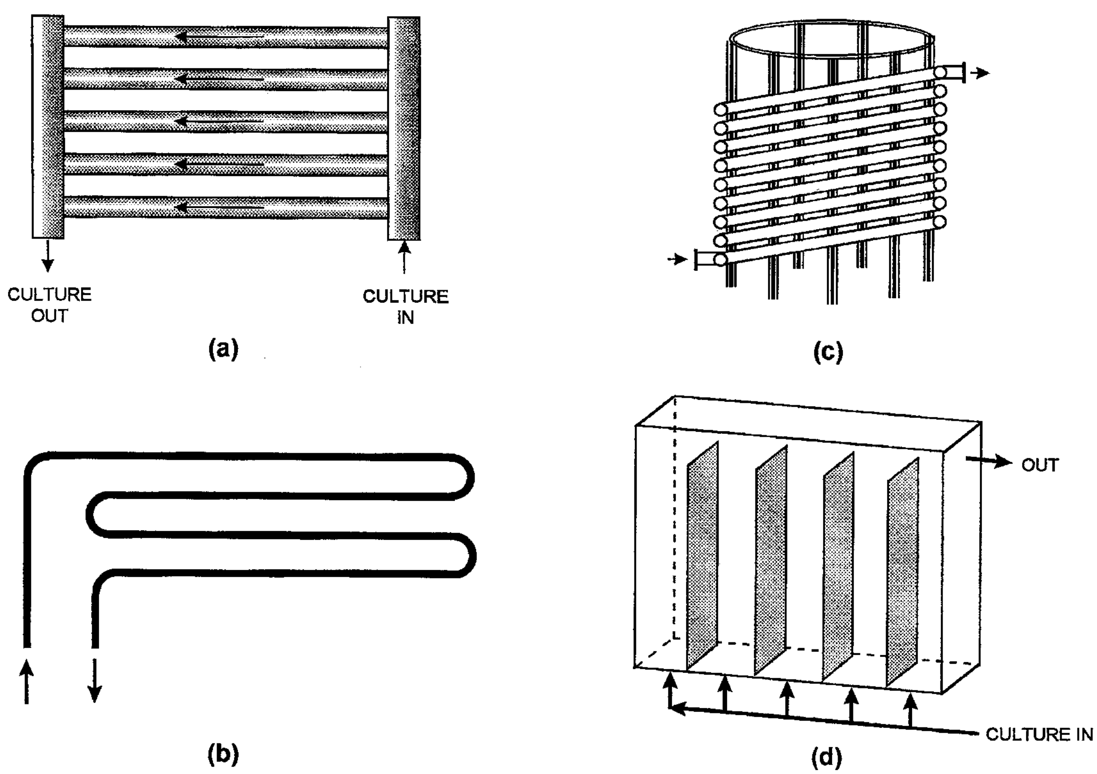


FIGURE 10 Light capture systems for photobioreactors: (a) tubular ladder, (b) continuous run serpentine, (c) biocoil, and (d) thin channels.

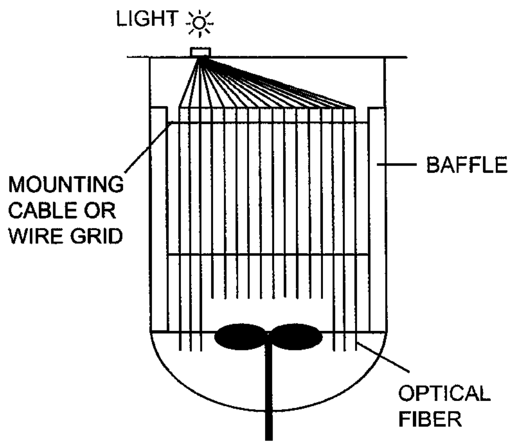


FIGURE 11 Illumination using optical fibers or waveguides.

Instead of using tubes, the photoreceiver is sometimes made of transparent plastic sheets, as in Fig. 10d. In other cases, a conventional vessel with a low surface-to-volume ratio may be illuminated by using optical fibers to convey light inside from an external source (Fig. 11), but this arrangement is not particularly effective. Irrespective of the design of the photoreceiver and the source of illumination (natural or artificial), light is generally the limiting nutrient in phototrophic culture. Except in optically dilute cultures, exponential growth does not persist for long in photosynthetic microbial culture. Because of light absorption and self-shading by cells in dense culture, light soon become limiting and growth kinetics change from exponential to linear. The depth related decline in light intensity is governed by the Beer–Lambert relationship, as follows:

$$\frac{I}{I_0} = \exp(-K_a XL), \qquad (1)$$

where I_0 is the incident light intensity, I is the intensity at depth L, X is the biomass concentration, K_a is the light absorption or extinction coefficient that depends on the pigment content of the cells, and L is the culture depth. Obviously, culture depth (i.e., tube diameter, channel depth) must remain quite shallow, or the local light intensity will become too low to support growth.

7. Other Bioreactor Configurations

The basic bioreactor configurations discussed above for heterotrophic growth (i.e., stirred tanks, bubble columns and airlift bioreactors, packed and fluidized beds) are generally satisfactory for a great majority of bioprocessing needs. In addition, some basic configurations have been especially adapted to better suite specific applications. For example, stirred vessels for animal and plant cell cultures employ different designs of impeller compared to ones used in typical microbial culture. Similarly, some animal cell culture vessels are installed with bundles of microporous polymer tubing for bubble-free oxygen supply to animal cells that are particularly susceptible to damage by bursting bubbles. Oxygen or a gas mixture containing oxygen flows through the tubing and oxygen diffuses into the culture broth through the liquid film held within pores in the tube wall. In other instances, a water-immiscible oxygen carrying liquid, or an oxygen vector (e.g., perfluorocarbons and silicone oils), is used to supply oxygen, as shown in Fig. 12. The carrier fluid is oxygenated in a sparged column by bubbling with air. The bubble-free carrier then circulates through the culture vessel where oxygen is transferred to the broth. The oxygen depleted carrier loaded with carbon dioxide returns to the aeration column (Fig. 12).

Other bioreactor designs include the rotating drum fermenter (Fig. 13) with internal baffles. This device is used to culture some suspended plant cells. The drum is filled to less than 40% of its volume and rotated on rollers for mixing. Another bioreactor configuration that is suitable for hairy root cultures of plants is the mist, spray, or fog bioreactor. The static root mass is contained in a chamber that is mostly empty. In this design, the nutrients are supplied as a mist of fine droplets suspended in circulating air currents that penetrate the spaces between the roots. The spray of nutrient solution is produced by using a compressed gas atomizer nozzle or a spinning disc spray device.

B. Solid-State Culture

Solid-state culture differs markedly from submerged culture. The substrates of solid-state fermentations are particulate solids that contain little or no free water. Steamed rice is a typical substrate. Beds of solids are difficult to agitate and solid-state fermentations do not employ intensive mixing. Small particles with large surface-to-volume ratios are preferred substrates because they present a larger surface for microbial action. However, particles that are too small, and shapes that pack together tightly (e.g., flat flakes, cubes), are undesired because close packing reduces interparticle voids that are essential for aeration. Similarly, too many fines in a batch of larger particles will fill up the voids. For fermentation, the substrate is loosely packed into shallow layers or heaps. Deep beds of substrate require forced aeration with moistened air. Aeration rates may vary widely; a typical range being (0.05–0.2) $\times$ 10^{-3} m^3 kg^{-1} min^{-1}. Occasional turning and mixing improve oxygen transfer, and reduce compaction and mycelial binding of substrate particles.

Unlike many submerged fermentations, solid-sate processes commonly use mixed cultures. Hygienic processing practices are followed in large industrial operations, but

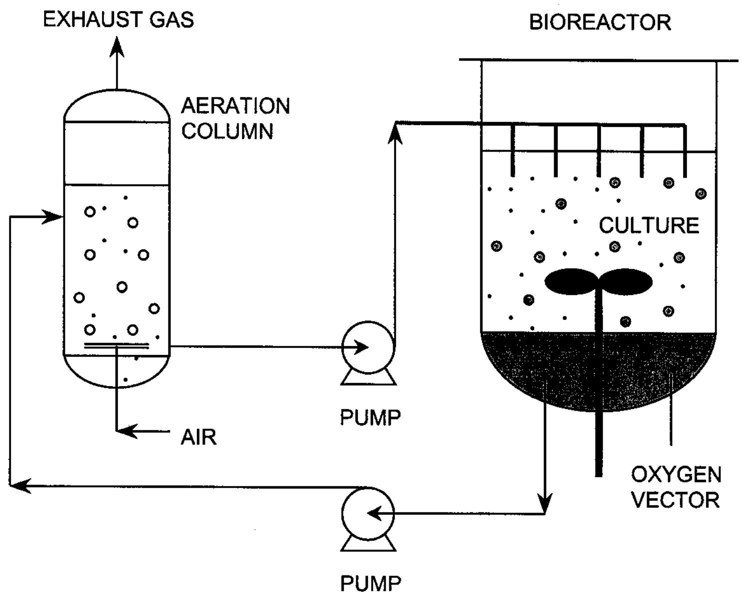


FIGURE 12 Bioreactor system for bubble-free aeration through a water-immiscible oxygen vector.

sterility standards that are common in submerged culture production of pharmaceuticals are not attained. Solid-state fermentation devices vary in technical sophistication from the very primitive banana leaf wrappings, bamboo baskets, and substrate heaps to the highly automated *koji* machines used mainly in Japan. Koji fermentations are widely practiced for making foods such as soy sauce. Koji, or molded grain, is a source of fungal enzymes that digest proteins, carbohydrates, and lipids into nutrients used by other microorganisms in subsequent fermentation. Koji comes in many varieties depending on the mold, the substrate, the method of preparation, and the stage of harvest.

Koji for soy sauce is made from soybeans and wheat. Soybeans, defatted soybean flakes, or grits are moistened and cooked in continuous pressure cookers. Cooked beans are mixed with roasted, cracked wheat. The mixed substrate is inoculated with a pure culture of *Aspergillus oryzae* (or *A. sojae*). The fungal spore density at inoculation is about 2.5×10^8/kg of wet solids. After a 3-day fermentation the substrate mass becomes green–yellow because of sporulation. Koji is now harvested for use in a second submerged fermentation step. Koji production is highly automated and continuous. Processes producing up to 4,150 kg h^{-1} koji have been described. Similar large-scale operations are used also to produce koji for miso and sake.

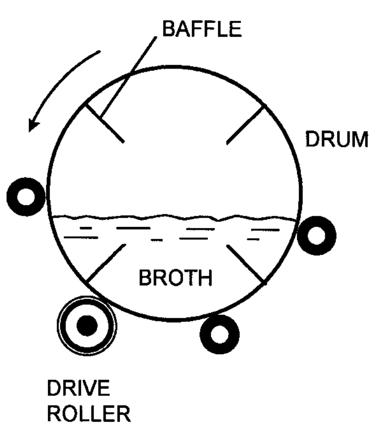


FIGURE 13 Rotating drum bioreactor for submerged culture.

Some common types of commercial solid-state fermenters are shown in Fig. 14. The *static bed fermenter* employs a single, static bed of substrate (e.g., steamed rice) located in an insulated chamber (Fig. 14a). The depth of the bed is usually less than 0.5 m. Oxygen is supplied by forced aeration through the substrate. The *tunnel fermenter* is an adaptation of the static bed device (Fig. 14b). Typically, the bed of solids is quite long, but again no deeper than 0.5 m. Tunnel fermenters may be highly automated with mechanisms for mixing, inoculation, continuous feeding, and harvest of substrate. The *agitated tank fermenter* uses one or more helical screw agitators mounted in a cylindrical or rectangular tank to agitate the fermenting substrate (Fig. 14c). The screws may move on horizontal rails. The *rotary drum fermenter* consists of a cylindrical drum that is supported on rollers (Fig. 14d). The rotation (1–5 rpm) of the drum causes a tumbling movement of the solids inside. Tumbling is aided by straight or curved baffles attached to the inside walls (Fig. 14d). Sometimes the drum may be inclined, causing the substrate to move from the higher inlet end to

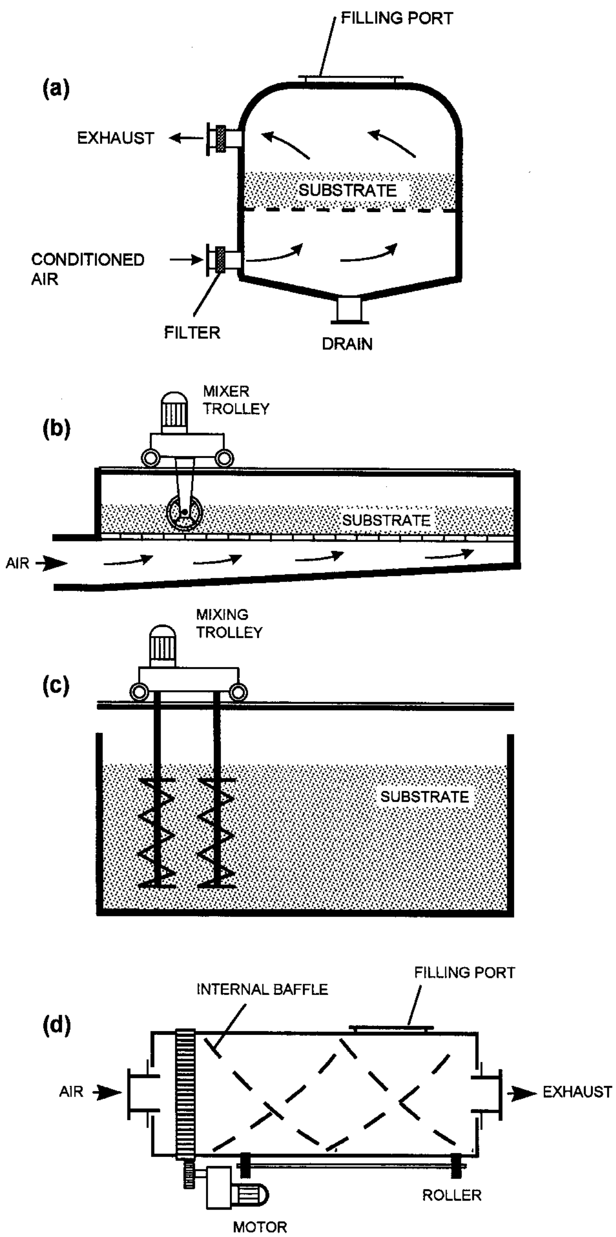


FIGURE 14 Bioreactors for solid-state fermentations: (a) static bed fermenter, (b) tunnel fermenter, (c) agitated tank fermenter, (d) rotary drum fermenter.

the lower outlet during rotation. Aeration is through the coaxial inlet and exhaust nozzles.

C. Bioreactors for Immobilized Enzymes and Cells

Immobilized enzyme and cell particles may be used in packed bed bioreactors, or the particles may be suspended in stirred tanks, bubble columns, airlift bioreactors, and fluidized beds, as discussed in an earlier section of this

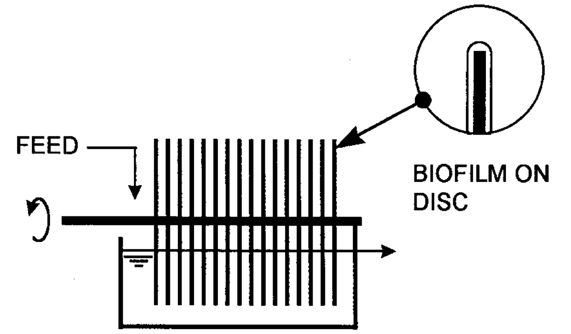


FIGURE 15 Rotating disc biofilm reactor. Microorganisms grow attached to the surfaces of the discs that rotate slowly to provide the cells with dissolved nutrients and oxygen.

article. Monolayers of animal cells anchored on small spherical microcarrier particles suspended in stirred bioreactors are widely used in producing viral vaccines (e.g., rabies and polio) and some therapeutic proteins. Similarly, suspended inert particles carrying microbial biofilms are employed in some wastewater treatment processes and other fermentations. Microbial biofilms growing on rotating discs (Fig. 15) are also used to treat wastewater. The discs, mounted on a shaft, slowly rotate through a pool of the water being treated. The dissolved pollutants in the water are taken up by the cells and degraded. The oxygen needed for the degradation diffuses into the biofilm as the disc cycles through the atmosphere.

Other bioreactor configurations have been developed specifically for immobilized enzymes and cells. Enzymes immobilized within polymeric membranes are used in hollow fiber (Fig. 16) and spiral membrane bioreactors (Fig. 17). In the hollow fiber device, many fibers are held in a shell-and-tube configuration (Fig. 16) and the reactant solution (or feed) flows inside the hollow fibers. The permeate that has passed through the porous walls of the fibers is collected on the shell side and contains the product of the enzymatic reaction. Also, instead of being immobilized in the fiber wall, enzymes bound to a soluble inert polymer may be held in solution that flows inside the hollow fiber. The soluble product of the reaction then passes through the fiber wall and is collected on the shell side; the enzyme molecule, sometimes linked to a soluble polymer, is too large to pass through the fiber wall.

Hollow fiber modules are sometimes used to culture animal cells that are confined to the shell side of the module; the separately oxygenated nutrient solution flows inside the fibers and perfuses the cells on the shell side. In the spiral membrane reactor (Fig. 17), the membrane that contains the immobilized enzyme is rolled into a spiral and confined within a shell. The feed or reactant solution flows in at one end and the product is removed from the opposite end of the cylindrical shell.

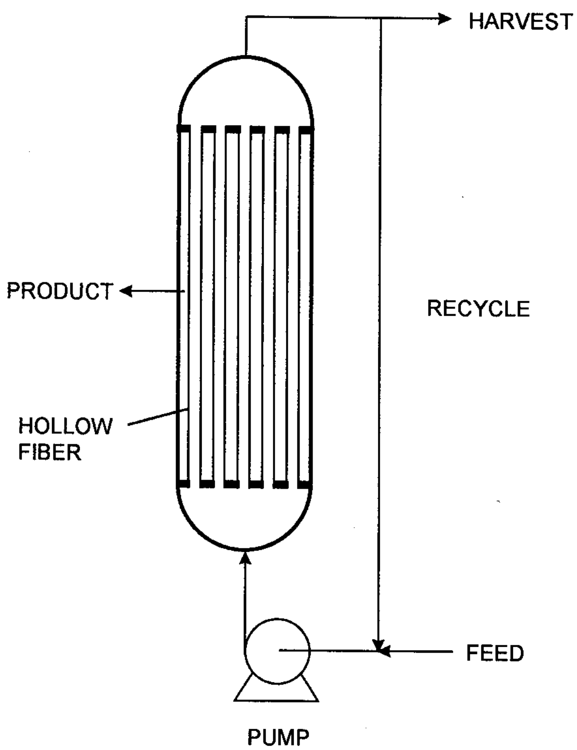


FIGURE 16 Hollow fiber membrane bioreactor.

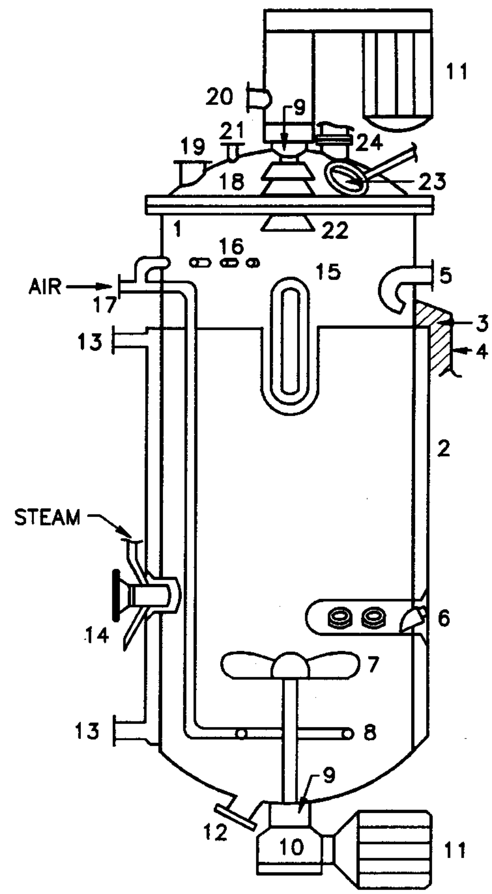


FIGURE 18 A typical submerged culture fermenter: (1) reactor vessel; (2) jacket; (3) insulation; (4) protective shroud; (5) inoculum connection; (6) ports for pH, temperature, and dissolved oxygen sensors; (7) agitator; (8) gas sparger; (9) mechanical seals; (10) reducing gearbox; (11) motor; (12) harvest nozzle; (13) jacket connections; (14) sample valve with steam connection; (15) sight glass; (16) connections for acid, alkali, and antifoam agents; (17) air inlet; (18) removable top; (19) medium feed nozzle; (20) air exhaust nozzle (connects to condenser, not shown); (21) instrumentation ports for foam sensor, pressure gauge, and other devices; (22) centrifugal foam breaker; (23) sight glass with light (not shown) and steam connection; (24) rupture disc nozzle.

III. CONSIDERATIONS FOR BIOREACTOR DESIGN

A. General Features

All bioreactors for monoseptic submerged culture have certain common features, as shown in Fig. 18. The reactor vessel is provided with side ports for pH, temperature, and dissolved oxygen sensors. Retractable sensors that can be replaced during operation are used commonly. Connections for acid and alkali (for pH control), antifoam agents, and inoculum are located above the broth level in the reactor vessel. The liquid level can be easily seen through a vertical sight glass located on the vessel's side (Fig. 18). A second sight glass is located on the top of the vessel and an externally mounted light can be used to illuminate the inside of the bioreactor. The sight glass on top can be internally cleaned by a jet of steam condensate. The vessel may be placed on a load cell to obtain a better indication of the amount of material it contains.

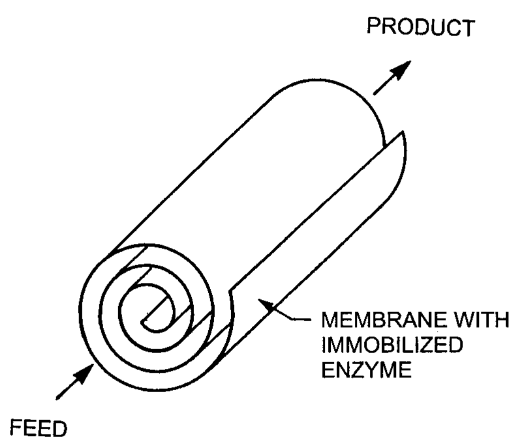


FIGURE 17 Spiral membrane bioreactor.

When mechanical agitation is used, either a top or bottom entering agitator may be employed. The bottom entry design is more common and it permits the use of a shorter agitator shaft, often eliminating the need for support bearings inside the vessel. The shaft of the agitator is provided with steam sterilizable single or double mechanical seals. Double seals are preferred, but they require lubrication with cooled clean steam condensate, or other sterile fluid. Alternatively, when torque limitations allow, magnetically coupled agitators may be used thereby eliminating the mechanical seals.

An air (or other gas mixture) sparger supplies oxygen (and sometimes carbon dioxide or ammonia for pH control) to the culture. Aeration of fermentation broth generates foam. Typically, 20 to 30% of the fermenter volume must be left empty to accommodate the foam and allow for gas disengagement. Foaming in bioreactors is controlled by a combination of chemical and mechanical methods. Chemical antifoaming agents are commonly mixed with the broth at initiation of fermentation. Further additions of antifoam agent are made from time to time, as needed. Typical antifoams are silicone oils, vegetable oils, and substances based on low molecular weight poly(propylene glycol) or poly(ethylene glycol). Emulsified antifoams are more effective because they disperse better in the fermenter. Excessive use of antifoams may interfere with some downstream separations such as membrane filtrations. Hydrophobic silicone antifoams are particularly troublesome, as they foul membrane filters and chromatography media. The use of antifoam chemicals is minimized by combining it with mechanical breakage of foam. A mechanical "foam breaker" may be installed in the headspace of the fermenter, as shown in Fig. 18. The device in Fig. 18 separates the foam—a dispersion of gas in liquid—into its components by centrifugal action, as explained in Fig. 19. The operation of the foam breaker and the addition of antifoam chemicals are controlled by signals from a foam sensor that extends into the bioreactor from the top. The shaft of the high-speed mechanical foam breaker must also be sealed using double mechanical seals as explained for the agitator.

In most instances, the bioreactor is designed for a maximum allowable working pressure of 3.78–4.10 bar (absolute pressure) at a design temperature of 150–180°C. The vessel is designed to withstand full vacuum. In North America the design conforms to the American Society of Mechanical Engineers (ASME), Section VIII, Division 1, *Boiler and Pressure Vessel Code*. Other codes may be acceptable in other locations. The reactor can be sterilized in place using saturated clean steam at a minimum pressure of 2.1 bar (absolute pressure). Overpressure protection is provided by a rupture disc located on top of the bioreactor. The rupture disc is piped to a contained drain. Usually a graphite burst disc is used because it does not crack or develop pinholes without failing completely. Other items located on the head plate of the vessel are nozzles for media or feed addition and for sensors (e.g., the foam electrode),

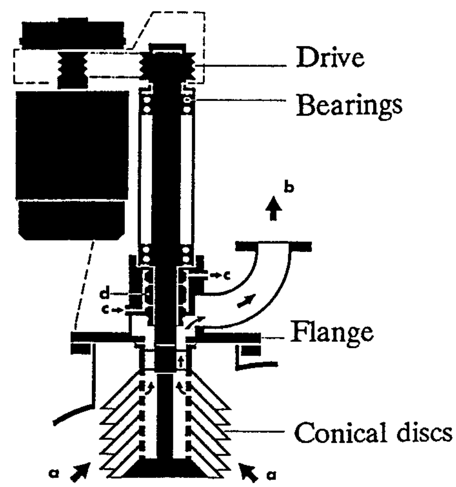


FIGURE 19 A mechanical foam breaker. The motor, drive, and shaft assembly are used to rotate the stack of conical discs at a high speed. The foam enters the spaces between the rotating discs at **a** and is separated into gas and liquid by the centrifugal force. The liquid spins into the bioreactor and liquid-free gas exhausts through the nozzle **b**. The mechanical seal **d** prevents leakage into and out of the sterile bioreactor. The seal is lubricated by sterile cooling water **c**.

and instruments (e.g., the pressure gauge). The vessel is designed to drain completely and a harvest nozzle is located at the lowest point on the reactor vessel (Fig. 18). The reactor is either provided with a manhole, or the top is removable. Flat head plates are commonly used in smaller vessels, but a domed construction of the head is less expensive for larger bioreactors (Fig. 18).

The bioreactor vessel should have few internals; the design should take into account the clean-in-place and sterilization-in-place needs. There should be a minimum number of ports, nozzles, connections, and other attachments consistent with the current and anticipated future needs of the process. The bioreactor should be free of crevices and stagnant areas where pockets of liquids and solids may accumulate. Attention to design of such apparently minor items as the gasket grooves is important. Easy to clean channels with rounded edges are preferred. As far as possible, welded joints should be used in preference to sanitary couplings. Steam connections should allow for complete displacement of all air pockets in the vessel and associated pipework, for sterilization. Evan the exterior of a bioprocess plant should be cleanly designed with smooth contours, minimum bare threads, and so forth.

The reactor vessel is invariably jacketed. In the absence of especial requirements, the jacket is designed to the same specifications as the vessel. The jacket is covered with chloride-free fiberglass insulation which is fully enclosed in a protective shroud as shown in Fig. 18. The jacket is provided with overpressure protection through a relief valve located on the jacket or its associated piping.

For a great majority of applications, austenitic stainless steels are the preferred material of construction for bioreactors. The bioreactor vessel is usually made in Type 316L stainless steel, while the less expensive Type 304 (or 304L) is used for the jacket, the insulation shroud, and other non-product contacting surfaces. The L grades of stainless steel contain less than 0.03% carbon, which reduces chromium carbide formation during welding and lowers the potential for later intergranular corrosion at the welds. The welds on internal parts should be ground flush with the internal surface and polished. Welds are difficult to notice in high-quality construction. In addition to the materials of construction, the surface finish also requires attention. The finish on surfaces which come in contact with the product material and, to some extent, the finish on external surfaces affects the ability to clean, sanitize, and sterilize the bioreactor and the general processing area. The surface finish has implications on stability and reactivity of the surface, and it may have process implications relating to microbial or animal cell adhesion to surfaces.

The mill-finished surface of stainless-steel sheet is unsatisfactory for use in bioreactors. Minimally, the surface should receive a mechanical polish. Mechanical polish is achieved by abrasive action of a sandpaper type material on metal. The surface finish may be specified by grit number, for example, 240-grit polish, which refers to the quantity of particles per square inch of the abrasive pad. The higher the grit number, the smoother the finish. More quantitative measures of surface finish rely on direct measurement of roughness in terms of "arithmetic mean roughness," Ra, or "root mean square roughness." Microscopic examination of even a highly smooth mechanically polished surface reveals a typical pattern of grooves and ridges that provide sites for microbial attachment. For example, a 320-grit polished surface will have an Ra of the order of 0.23–0.30 μm. Hence, for internal surfaces of bioreactors, electropolished surface is preferable to mechanical polish alone.

Electropolishing is an electrolytic process which preferentially removes the sharp microscopic surface projections arising from mechanical polishing; the result is a much smoother finish. Electropolishing significantly reduces the metal surface area and, hence, the product-metal contact area. The treatment imparts corrosion resistance to stainless steel by removing microscopic regions of high local stress; it creates a passivated steel surface, rich in protective chromium oxide. To attain a suitable electropolished finish, the surface should be previously mechanically polished; however, there is little advantage to starting with a much better than 220-grit (Ra $\approx$ 0.4–0.5 μm) polished surface. If mechanical polish alone must be used, it should be at least 240 grit, and the direction of polish should be controlled to produce a vertical grain for good drainage. The surface should receive a nitric acid wash treatment as a minimum. The orientation of the grain does not seem to be of consequence if the surface is to be electropolished.

B. Mixing, Heat, and Mass Transfer

1. Mixing and Shear Effects

A minimal intensity of mixing is required in a bioreactor to suspended the biocatalyst and substrate particles, prevent development of pH and temperature gradients in the bulk fluid, and improve heat and mass transfer. Mixing also enhances transfer of nutrients and substrates from the fluid to the biocatalyst particles and helps remove and dilute inhibitory metabolites that may be produced. Mixing is generally provided by mechanical agitation or by bubbling compressed gas into the fluid. Excessively intense mixing is harmful; too much turbulence damages certain cells, disintegrates immobilized biocatalyst pellets, and may dislodge biofilms from carriers. Freely suspended microorganisms are generally tolerant of hydrodynamic forces (or "shear" forces) encountered in bioreactors under typical conditions of operation; however, animal cells, suspended plant cells, certain microalgae, and protozoa are especially prone to shear damage. Forces associated with rupture of sparged gas bubbles are known to destroy animal cells. Damage is minimized by using lower aeration velocities, larger bubbles (diameter $\geq$ 0.01 m), and supplementation of the culture medium with protective additives such as the surfactant Pluronic F68. Aeration associated power input in bioreactors for animal cell culture is typically kept at less than 50 W m^{-3}. The power input may be calculated as follows:

$$\frac{P_G}{V_L} = \rho_L g U_G, \tag{2}$$

where P_G is the power input, V_L is the volume in the bioreactor, ρ_L is the density of the broth, g is the gravitational acceleration, and U_G is the superficial gas velocity. The velocity is calculated as the volume flow rate of the gas divided by the cross sectional area of the bioreactor.

2. Oxygen Supply and Carbon Dioxide Removal

Animal and plant cells need oxygen to survive. Many microorganisms require oxygen (i.e., they are *obligate aerobes*) but oxygen may be toxic to others (*anaerobes*). Some microbes may switch between aerobic and anaerobic growth and are said to be *facultative*. Sufficiency of oxygen supply is necessary to prevent growth limitation in aerobic cultures. Oxygen is provided usually by sparging the broth with air or some other oxygen-containing mixture of gases. Other specialized methods of oxygen supply are used in a few bioprocesses (e.g., Fig. 12).

Oxygen is sparingly soluble in aqueous broths and even a short interruption in aeration rate in some microbial fermentations may produce anaerobic conditions that may potentially damage the cells. The precise oxygen requirements of a fermentation process depend on the microorganism, the degree of oxidation of the substrate being used for growth, and the rate of oxidation. Oxygen becomes hard to supply when the demand exceeds 4–5 kg O_2 m^{-3} hr^{-1}. Many microbial fermentation broths are highly viscous and difficult to mix. This further complicates the transfer of oxygen from the gas phase to the broth. Once the concentration of dissolved oxygen falls below a critical value, the microbial growth becomes limited by oxygen. The critical dissolved oxygen concentration depends on the conditions of culture and the microbial species. Under typical culture conditions, fungi such as *Penicillium chrysogenum* and *Aspergillus oryzae* have a critical dissolved oxygen value of about 3.2×10^{-4} kg m^{-3}. For baker's yeast and *Escherichia coli*, the critical dissolved oxygen values are 6.4×10^{-5} and 12.8×10^{-5} kg m^{-3}, respectively.

Animal and plant cell cultures have lower oxygen demands than microbial cells. Oxygen consumption rates for animal cells are in the range of 0.05–0.5 mmol/10^9 cells per hr. In batch suspension culture of animal cells, the maximum cell concentration typically does not exceed 2×10^6 cells ml^{-1}. Higher concentrations, $>10^7$ cells ml^{-1}, are attained in perfusion culture without cell retention. Perfused culture with cell retention permits cell densities of around 10^9 cells ml^{-1}. *In vitro* cultured animal cells are generally tolerant of high concentrations of dissolved oxygen, e.g., up to 100% of air saturation; however, the optimal concentration is about 50% of air saturation but may vary with cell type. Oxygen concentrations of $>100\%$ of air saturation have been associated with oxidative damage to cells whereas concentrations $\leq 0.5\%$ of air saturation inhibit the TCA cycle and lead to an enhanced production of lactate. Concentrations $<10\%$ of air saturation will limit growth of some cells, but for others oxygen limitation is encountered around 0.5% of air saturation. Plant cells consume oxygen at a rate of (3–15) $\times 10^{-5}$ mol kg^{-1} DW s^{-1} and the maximum cell density in suspension culture tends to be 20–30 gDW L^{-1}.

As with dissolved oxygen, concentration of dissolved carbon dioxide influences microbial and cell growth. Too much carbon dioxide is detrimental to most aerobic fermentations but cultures of photosynthesizing microbes may require carbon dioxide in the aeration gas. Also, carbon dioxide is added at roughly 5% (by vol) to gas mixture used in sparging animal cell cultures when the culture broth is buffered with carbonate-bicarbonate system. Normal mammalian cells need carbon dioxide as a substrate for carboxylation of pyruvate to oxaloacetic acid, but established cell line may not require carbon dioxide.

3. Heat Removal and Temperature Control

All fermentations generate heat. In submerged cultures, 3–15 kW m^{-3} of the heat output typically comes from microbial activity. In addition, mechanical agitation of the broth produces up to 15 kW m^{-3}. Consequently, a fermenter must be cooled to prevent temperature rise and damage to culture. Temperature is controlled by circulating cooling water in a jacket that surrounds the bioreactor vessel (Fig. 20a). In addition to the jacket, an internal cooling coil (Fig. 20b) becomes necessary in large bioreactors. In some cases, cooling is achieved by recirculating the broth through an external heat exchanger (Fig. 20c). In small vessels, a "ringlet" coil (Fig. 20d) that enters the vessel through a large port on top, may provide sufficient cooling. Heat removal tends to be difficult because, typically, the temperature of the cooling water is only a few degrees lower than that of the fermentation broth. Industrial fermentations are commonly limited by the heat transfer capability. The ability to remove heat depends on the surface area available for heat exchange, the temperature difference between the broth and the cooling water, the properties of the broth and the coolant, and the turbulence in those fluids. The geometry of the fermenter determines the heat exchange area that can be provided.

Because metabolic heat generation depends on the oxygen consumption rate, heat removal in large vessels

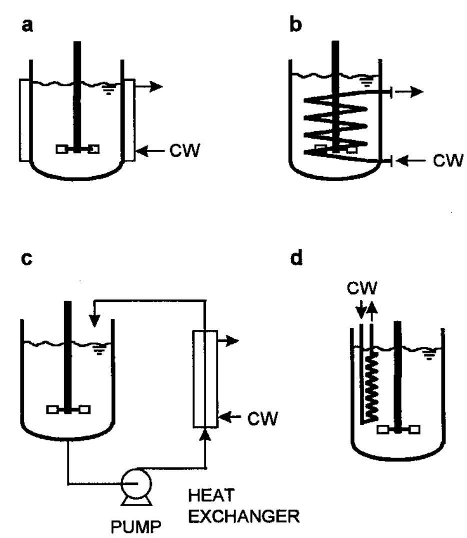


FIGURE 20 Heat-exchange methods for bioreactors: (a) jacket, (b) full internal coil, (c) recirculation of broth through an external heat exchanger, (d) ringlet coil. Cooling water (CW) enters and exits the heat exchange devices, as shown.

becomes difficult as oxygen consumption rate approaches $5\ \mathrm{kg\ m^{-3}\ h^{-1}}$. About 0.54 kJ of heat is generated for each millimole of oxygen consumed. Oxygen consumption rate in microbial broths typically ranges from 2×10^{-4} to $1 \times 10^{-3}\ \mathrm{kg\ m^{-3}\ s^{-1}}$. Typically, microorganisms growing on hexose sugars such as glucose produce about 10.9 MJ of heat per kilogram of biomass formed. For cells growing on highly reduced substrates such as hydrocarbons, heat generation is greater, about 28.5 MJ/kg of biomass produced. These figures are for fermentations in which biomass is the only product. Most microbial and plant cells are cultured at a temperature of between 20 and 30°C. Mammalian cells are usually grown at 37°C. Insect cells prefer a lower temperature, e.g., 26°C. Temperatures of greater than 40°C are optimal for certain *thermophilic* microorganisms.

In addition to removing the metabolic heat, a fermenter must provide for heat transfer during sterilization and subsequent cooling. Liquid (or slurried) fermentation medium for a batch fermentation may be sterilized using batch or continuous processes. With batch processes, the medium or some of its components and the fermenter are commonly sterilized together in a single step by heating the dissolved or slurried medium inside the fermenter. For *in situ* sterilization, steam may be injected directly into the medium or heating may be through the fermenter wall.

High temperature (typically 121°C) heating during sterilization often leads to undesirable reactions between components of the medium. Such reactions reduce yield by destroying nutrients or by generating growth inhibitory compounds. This thermal damage is prevented or reduced if only certain components of the medium are sterilized in the fermenter and other separately sterilized components are added later. Sugars and nitrogen-containing components are often sterilized separately. Dissolved nutrients that are especially susceptible to thermal degradation may be sterilized by passing through hydrophilic polymer filters that are rated to retain particles down to 0.45 μm. Even finer filters (e.g., 0.2 μm rated particle retention) are available.

Heating and cooling of a large fermentation batch takes time that ties up a fermenter unproductively. In addition, the longer a medium remains at high temperature, the greater is the thermal degradation or nutrient loss. Therefore, continuous sterilization of the culture medium into a presterilized fermenter is preferable even for batch fermentations. Continuous sterilization is rapid and it limits nutrient loss; however, the initial capital expense is greater because a separate sterilizer is necessary.

A heat exchange system must be able to handle the high heat loads encountered during sterilization by steam and subsequent cooling. Irrespective of the specific heat exchange system used (Fig. 20), the operational details can be quite complex as shown in Fig. 21 for a jacketed bioreactor. Valves 1, 10, and 5 in Fig. 21 are control valves that modulate the flow of steam and cooling water; valves 2 and 9 are vacuum breakers that allow the pipework to drain under gravity; valve 3 is a pressure relief valve to protect the jacket and the pipework against pressurization above the safe acceptable limit; and all other valves are pneumatically operated devices that are either fully open or fully closed.

For sterilization the jacket is heated by steam. Valves 4–6, 8, and 10 are closed and the pump 7 is off. Valve 11 is opened to drain the jacket. After a short period, valve 11 is closed and valve 12 is opened. Valve 1 is opened to let steam into the jacket. The condensate drains through the steam trap 13. The temperature and pressure of the circuit are monitored at TIC1 and PI1. Cooling is carried out by closing valves 12 and 1; valves 8 and 4 are opened. Cooling water enters the circuit at valve 4, flows up the jacket and out to the cooling water return line (Fig. 21). All operations are generally automated for consistent and error-free control.

Animal cell culture may actually require some warming to maintain the temperature at 37°C. The circuit shown in Fig. 21 uses a closed recirculation loop to control the temperature. During culture, valves 8 and 6 are opened and the pump 7 is turned on. The water is pumped through a compact plate heat exchanger (PHE) where it is indirectly heated by controlled (control valve 10) flow of steam. The heated water now passes the temperature indicator/controller TIC1 and enters the jacket. The water recirculates via valve 8 and pump 7. Cold water is injected (control valve 5) in the circuit to maintain the temperature. Any excess water leaves the loop via the relief valve 3. In some designs, direct steam injection into the circulating loop may be used instead of the heat exchanger. In small fermenters, the exchanger may be replaced by electric heating. Notice the flexible connections between the fermenter and the temperature control pipework (Fig. 21). These connections are necessary for fermenters that rest on load cells (for weight measurement); the connections allow the fermenter to move freely.

Compared to submerged culture, biomass levels in solid state fermentations are lower at 10–30 $\mathrm{kg\ m^{-3}}$. Nevertheless, because there is little water and the density of the substrate is relatively small, the heat generation per unit fermenting mass tends to be much greater in solid state fermentations than in submerged culture. Temperature can rise rapidly, again, because there is little water to absorb the heat. Cumulative metabolic heat generation in koji fermentations for a variety of products has been noted at 419–2387 $\mathrm{kJ\ kg^{-1}}$ solids. Higher values, up to 13,398 $\mathrm{kJ\ kg^{-1}}$, have been observed during composting. Peak heat generation rates in koji processes range over 71–159 $\mathrm{kJ\ kg^{-1}\ hr^{-1}}$, but the average rates are more moderate at

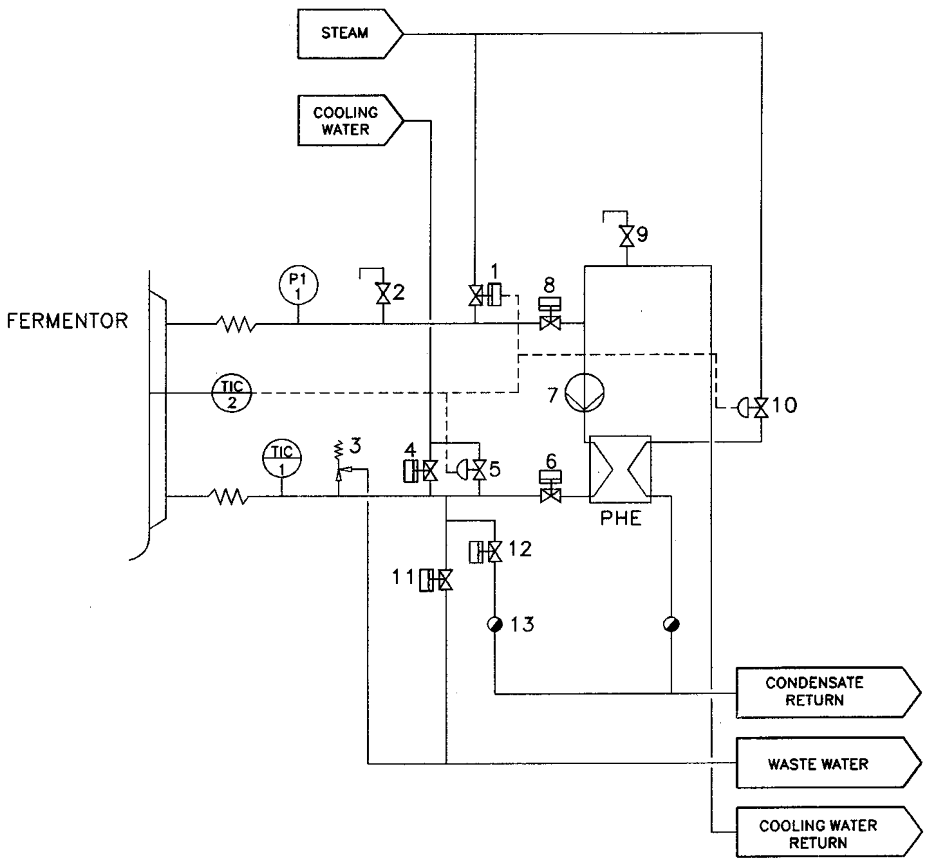


FIGURE 21 Temperature control circuit on a bioreactor.

25–67 kJ kg^{-1} hr^{-1}. Peak metabolic heat production rate during fermentation of readily oxidized substrates such as starch can be much greater than in typical koji processes.

In solid-state fermentation the substrate temperature is controlled mostly through evaporative cooling; drier air provides a better cooling effect. Intermittent spray of cool water is sometimes necessary to prevent dehydration of the substrate. Air temperature and humidity are also controlled. Occasionally, the substrate-containing metal trays may be additionally cooled by circulating a coolant, even though most relatively dry and porous substrates are poor conductors of heat. Intermittent agitation of substrate heaps further aids heat removal. Despite much effort, temperature gradients in the substrate do occur, particularly during peak growth.

C. Monoseptic Operation, Cleaning, Sterilization

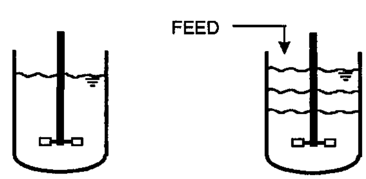
Many commercial bioprocesses utilize only pure cultures. Maintenance of monoculture is vital to success of such processes; hence, a bioreactor must be sterilized prior to inoculation and contamination during operation must be prevented. A contaminating virus or microbe may destroy the culture, reduce productivity, or lead to other unwanted results. Poor design and operation of a bioreactor increase the chance of contamination and cause financial loss.

Sterilization with steam at a minimum temperature of 121°C is the norm. The sterilization temperature must be attained everywhere in the vessel and continuously held at the requisite value for at least 20 min. After sterilization and during culture the contents of the bioreactor remain fully isolated from the external environment. Air enters the vessel through presterilized hydrophobic membrane filters that prevent ingress of contaminating microbes. Similar filters are located on the air exhaust pipe. These filters are rated for removing particles down to 0.45 μm, or even 0.1 μm. The bioreactor may be sterilized together with the culture medium, or separately. In the latter case, once the bioreactor has cooled, the medium is pumped in through a sterilizing filter that removes any contaminating microbes. Alternatively, the medium is steam sterilized, cooled, and then transferred to the bioreactor through pipework that is fully isolated from the surroundings.

After the culture and before next sterilization, the bioreactor must be thoroughly cleaned usually by automated clean-in-place methods. In-place cleaning does not require dismantling the bioreactor and saves time. Automation assures consistency of cleaning. Cleaning between batches is essential to preventing cross-contamination of products. Also, a clean bioreactor is easier to sterilize. Cleaning is achieved by physical action of high velocity flow, jet sprays, agitation, and chemical action of cleaning agents enhanced by heat. While mechanical forces are necessary to remove gross soil and to ensure adequate penetration of cleaning solutions to all areas, most of the cleaning action is provided by chemicals—surfactants, acids, alkalis, and sanitizers. A generally applicable cleaning scheme for bioreactors utilizes a water pre-rinse to remove gross soil; a hot alkali recirculation step to digest and dissolve away the remaining soil; and a water wash to remove residual alkali. A bioreactor that processes injectable drugs, should be rinsed with a hot water-for-injection (WFI) wash as the final cleaning step. Optional acid wash and sanitization steps may be added in some applications.

D. Operational Modes of Bioreactors

The way a culture is operated and fed has a profound impact on the outcome of the fermentation. Also, the reactant feeding and reactor operational strategies affect performance of enzyme bioreactors and those that use non-viable biomass as a biocatalyst. Most bioreactors for microbial growth and culture of other cells are operated as *batch* and *fed-batch* devices. A batch fermentation is initiated by inoculating a presterilized and cooled medium that is contained typically in a well-mixed bioreactor (Fig. 22a). The medium composition in the fermenter changes continuously as nutrients are consumed to produce biomass and metabolites. The broth is harvested after the designated batch time. The broth volume in a batch fermenter remains essentially constant (Fig. 22a), discounting any evaporative loss. A fed-batch culture is identical to a batch operation, except that a feed is added to the broth continuously or intermittently (Fig. 23b). The volume of the broth increases with time. Also, the feeding rate generally incenses with time, to satisfy the demand of an exponentially increasing cell population. The broth is harvested at the end of the batch period.



(a) (b)

FIGURE 22 Bioreactor operational modes: (a) a well-mixed batch bioreactor, (b) a well-mixed fed-batch bioreactor.

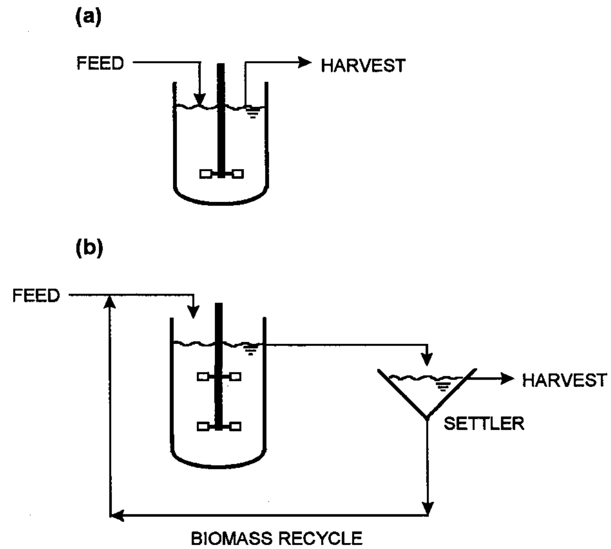


FIGURE 23 Bioreactor operational modes: (a) a continuous flow well-mixed bioreactor, (b) a continuous flow well-mixed bioreactor with biomass recycle.

In a *well-mixed steady-state continuos culture* (Fig. 23a), the broth composition does not vary with time and position in the bioreactor. Typically, a continuous fermentation starts as a batch culture and switches to continuous feeding when a sufficient concentration of biomass has been obtained. The feed is added continuously and at a constant rate. The broth volume does not change, as the rate of harvest matches the feeding rate (Fig. 23a). Sometimes, a well-mixed continuos culture may be carried out with recycle of a part of the harvested biomass to the bioreactor (Fig. 23b). This strategy increases the steady state biomass concentration in the reactor, improves conversion of the substrate, and enhances the productivity. Some continuous flow reaction and production schemes use a number of well-mixed reactors in series (Fig. 24a–c). The harvest of one reactor becomes the feed for the next. This arrangement is especially useful when different environmental conditions are needed at different stages of a process, for example, when the requirements for growing the biomass differ from the ones for synthesis of a metabolite by the cells. A multistage continuous array of well-mixed reactors may be fed with different feeds and precursor compounds at different stages, as shown in Fig. 24b. Also, such a series of reactors my employ recycle of the biomass (Fig. 24c) to the first stage, or one or more of the other stages.

Continuous flow processes sometimes use a *plug flow* bioreactor in which there is little mixing of fluid elements in the direction of flow (Fig. 25a). This type of flow is typically achieved in long tubes and channels. The composition of the broth does not change with time at a fixed

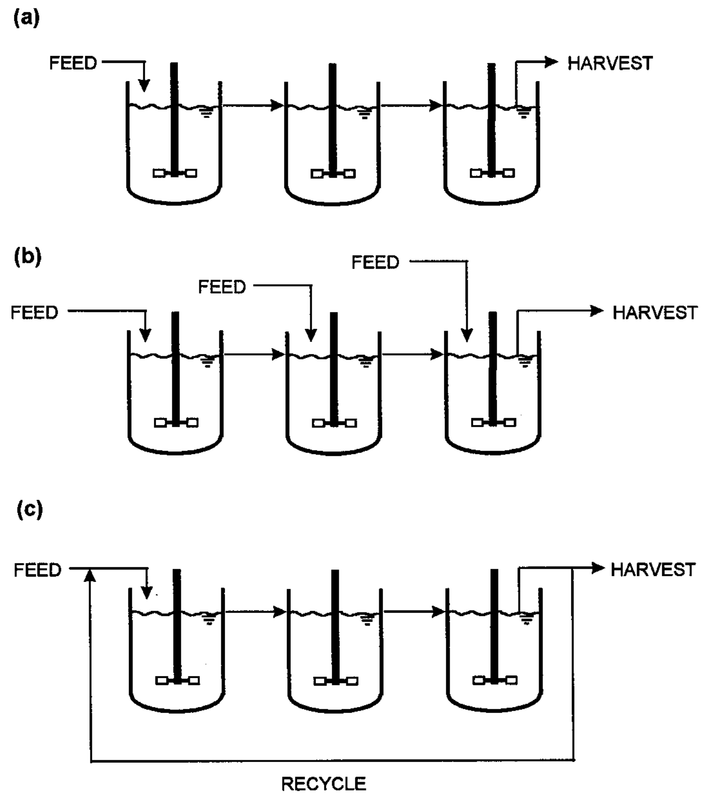


FIGURE 24 Bioreactor operational modes: (a) a series of continuous flow well-mixed bioreactors, (b) use of different feeds at different stages in a series of continuous flow well-mixed bioreactors, (c) a series of continuous flow well-mixed reactors with biomass recycle.

location in a plug flow bioreactor operating at steady state; however, the composition changes with position in the direction of flow. A plug flow bioreactor behaves the same as a series of many continuous flow well-mixed bioreactors (Fig. 24a). In terms of kinetics, a plug flow bioreactor is equivalent to a batch fermenter having the same residence time (i.e., the total time spent by the broth in the bioreactor) as in the plug flow device. A plug flow bioreactor for biomass production must be inoculated continuously, or a portion of the harvested biomass must be recycled to the inlet of the bioreactor (Fig. 25b), to prevent wash out of the culture by the sterile feed. Multipoint feeding is sometimes used in plug flow bioreactors, as shown in Fig. 25c.

Continuous flow operation of a bioreactor such that the cells are recycled or retained within the reactor, is sometimes known as perfusion culture. Many different kinds of devices are available for cell retention and recycle. These devices may be located within or outside the bioreactor vessel. External sedimentation tanks (Fig. 23b) are often used to recycle biomass in wastewater treatment processes. External and internal enhanced-rate sedimentors are also employed in animal cell culture bioreactors, as shown in Fig. 26a, b. Because of the small differences in density and diameter of the viable and nonviable animal cells, the inclined channel sedimentor (Fig. 26b) may preferentially retain viable cells in the bioreactor while allowing many of the nonviable ones to wash out. Another device that is commonly deployed for partial retention of animal cells is the "spinfilter" shown in Fig. 27. A spinfilter

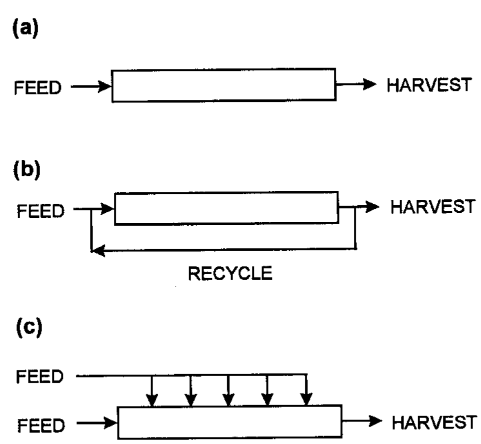


FIGURE 25 Bioreactor operational modes: (a) a continuous plug flow bioreactor, (b) a continuous plug flow bioreactor with biomass recycle, (c) multipoint feeding of a plug flow bioreactor.

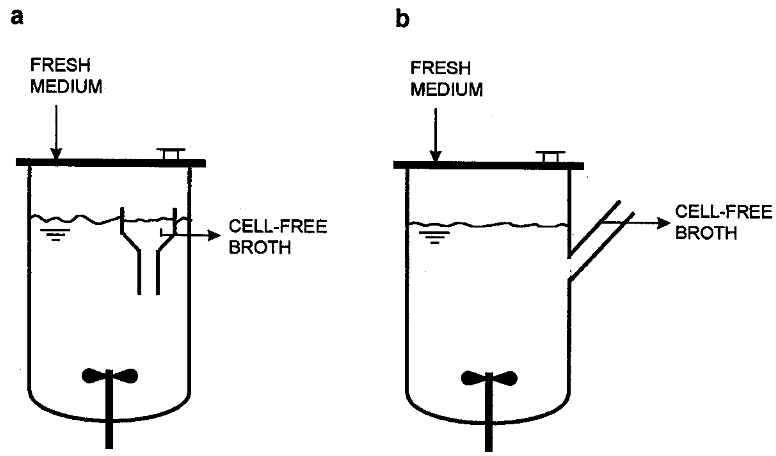


FIGURE 26 Cell retention by sedimentation in perfusion culture: (a) internal sedimentor, (b) inclined channel sedimentor.

is a cylinder formed of wire meshing. The openings of the wire screen are significantly larger (e.g., 25 μm) than the cells which are retained by a hydrodynamic mechanism requiring rapid rotation (e.g., 500 rpm) of the spinfilter. The mostly cell-free spent medium is withdrawn from the zone within the rotating screen. Depending on the specifics of design and operation, a spinfilter may preferentially retain the viable cells. Also, a noninvasive acoustic sedimentation device has been developed and this is installed on the outside of the bioreactor harvest pipe. This device uses sound waves to aggregate the cells which sediment back into the bioreactor vessel.

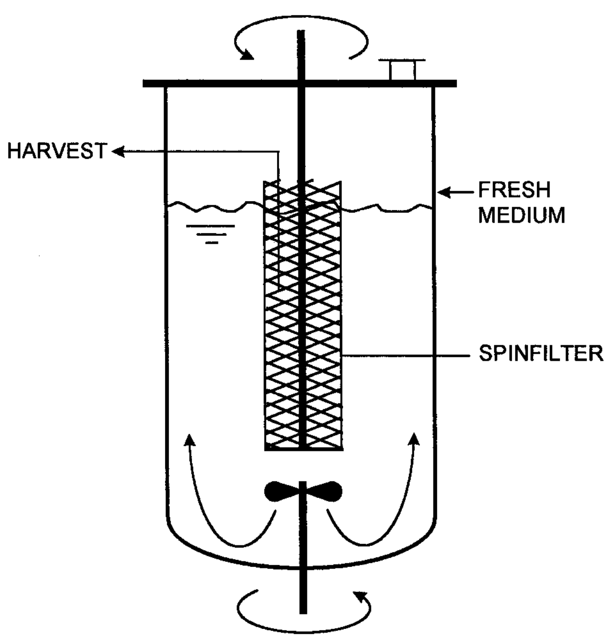


FIGURE 27 A spinfilter device of cell retention in perfusion culture of animal cells.

Spinfilters and sedimentation devices do not retain all cells within a bioreactor and some washout occurs. Total retention of biomass is feasible by using an external microfilter to continuously harvest a cell-free stream, as shown in Fig. 28. The cells are returned to the bioreactor. The bioreactor operational schemes identified here are the ones most commonly used; other variations on these schemes are encountered occasionally.

E. Medium Composition

The composition of the nutrient medium used to grow cells determines the rate of production, the type of products produced, and the yield of biomass and products. The medium also influences the cost of production, the quality of the product, and factors such as the broth rheology. The medium must provide all the elements that compose the product and the biomass. These elements (e.g., C, N, O, S, P) must be provided in a suitable form and ratios that are designed to achieve specific effects. The growing cells may require additional complex organic molecules (micronutrients) that they are unable to synthesize but that are essential to growth. In addition, the medium may contain specific precursor compounds, the substrates that are being biotransformed, and inhibitors or inducers of specific enzymes. While the medium needs many components, care is necessary to prevent contamination with others that may affect the process performance. For example, presence of iron in *Aspergillus niger* fermentation broth greatly suppresses the production of citric acid. Proper formulation of the medium is also important in cell-free enzyme reactors, where the rate of reaction and the extent of substrate (or/and product) inhibition depend on the composition of the reaction medium. Some enzyme reactions are carried out in organic solvents containing small amounts of water.

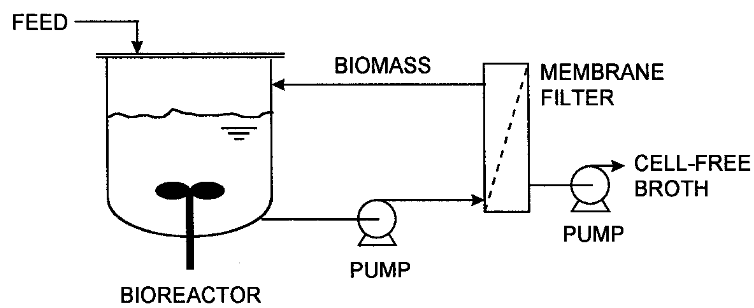


FIGURE 28 Total recycle of biomass by using a microfiltration device.

Medium design for viable culture needs to consider the osmotic pressure of the broth as an important variable.

Osmotic pressure of a solution is a measure of the concentration of dissolved molecules and ions present as individual particles. All cells are susceptible to osmotic pressure of the surrounding medium, but cells without walls (e.g., animal cells, protoplasts) are especially susceptible to osmotic stress related damage. When the suspending fluid has a higher osmotic pressure than the cells, water is drawn out of the cells and it may become so dehydrated that metabolism ceases. Incidentally, this is why high salt and high sugar media often have a preservative action, e.g. in pickling of vegetables. When the osmotic pressure is low compared to that in the cell, the cell takes up water and may burst. Osmotic shock produced by rapid dilution is sometimes used to rupture cells, especially animal cells. Osmotic pressure also influences plant cell suspension culture.

Media formulations do not generally specify the osmotic pressure; instead, the number of dissolved particles is given as osmolarity or osmolality. Osmolality is the number of moles of particles per kilogram of solution whereas osmolarity is the number of moles per liter of solution. One mole of particles is an osmole, abbreviated as Osm. Animal cell culture media have an osmolality of 280 to 320 mOsm kg^{-1} to conform to the osmolality of serum (290 mOsm kg^{-1}). Osmolality is not easily calculated especially when a medium has many components and the degree of dissociation is not known. In such cases, osmolality is estimated from measurements of freezing point depression and other colligative properties (i.e., those dependent on the concentration of dissolved particles). In cell culture media, sodium chloride is used for adjusting osmolality to the requisite value.

F. Kinetics, Productivity, and Bioreactors

Design and performance analysis of a bioreactor are inseparably linked with the kinetics of the bioreaction for which the reactor is intended. Some common bioreactions include growth of microorganisms and other cells, and reactions involving cell-free enzymes. Essential aspects of kinetics for bioreactor design are discussed here.

1. Cell Growth

Once a properly formulated and sterilized medium has been inoculated with a *seed culture* or inoculum, the cells grow and multiply. In a batch culture, the cell or biomass concentration increases with time as shown in Fig. 29, which is typical. A short *lag phase* of little or no growth is followed by a period of *exponential growth*. The lag phase is an adaptation period in which the cells become acclimatized to a new environment. The length of the relatively unproductive lag phase may be shortened by increasing the inoculum size and ensuring that the growth environment (medium, pH, temperature, etc.) in the bioreactor is the same as the one in which the inoculum was grown. Typically, the inoculum should be in the late exponential phase of growth. The volume of a microbial inoculum should between 5 and 10 percent of the volume of the medium being inoculated. Larger inocula are needed for slower growing cells such as animal and plant cells. In cultivation of animal cells, the inoculum size is selected to provide an initial cell count of (2–4) $\times\ 10^5$ cells/ml. After the lag period, the exponential growth persists usually until an essential nutrient (the growth limiting nutrient or substrate) runs low in the medium, or until some inhibitory product of metabolism accumulates to a growth inhibitory concentration. The cells then enter a phase of zero net growth, or the *stationary phase* (Fig. 29). This is followed by a *decline phase* or death phase in which there is a net loss of biomass as cells die and lyse. Usually an attempt is made to achieve a maximum growth rate and maintain it for as long as possible.

The biomass growth rate in a bioreactor, i.e., the change in biomass concentration with time, or dX/dt, depends on the viable biomass concentration X present at any time. In other words, growth is self catalyzing, or *autocatalytic*. In exponential growth, the growth rate is expressed as follows:

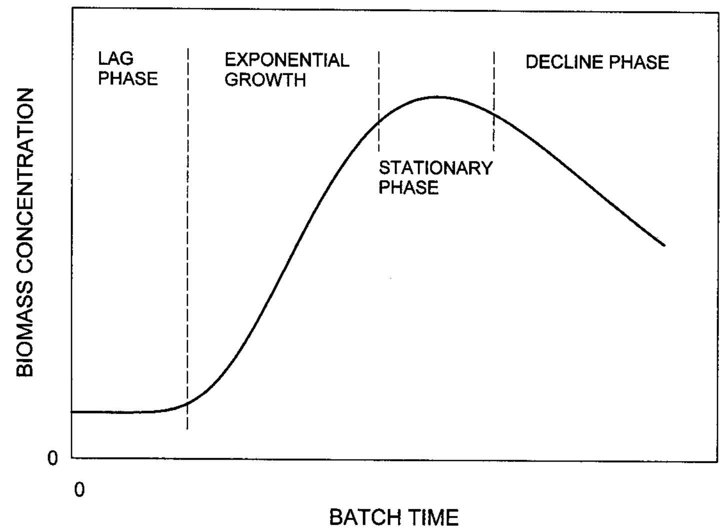


FIGURE 29 A typical biomass growth profile in batch culture.

$$\frac{dX}{dt} = \mu X, \tag{3}$$

where μ is a constant known as the *specific growth rate.* Equation (3) may be written in its integrated form, as follows:

$$\ln \frac{X}{X_o} = \mu t, \tag{4}$$

where X_o is the initial biomass concentration at any time $t = 0$ and X is the concentration at time t. The time to double the biomass concentration, or the *doubling time* t_d, may be estimated from Eq. (4) by substituting t_d for t and $2X_o$ for X_o; thus,

$$t_d = \frac{\ln 2}{\mu}. \tag{5}$$

Some typical values of the doubling time are noted in Table I for the various kinds of microbial and other cells.

In steady-state continuous culture in which the cells experience an unchanging environment, the specific growth rate depends on the concentration S of a *growth limiting substrate.* This dependence is generally described by Monod kinetics, as follows:

TABLE I Typical Doubling Times

Cell type	t_d (min)
Bacteria	20–45
Yeasts	90
Molds	160
Protozoa	360
Hybridomas	630–1260
Plant cells	3600–6600

$$\mu = \frac{\mu_{\max} S}{K_s + S}, \tag{6}$$

where $\mu_{\max}$ is the *maximum specific growth rate* and K_s is the value of S at which the specific growth rate is half of its maximum value. K_s is known as the *saturation constant.* The specific growth rate increases with increasing substrate concentration in a hyperbolic manner, as shown by the solid line in Fig. 30. The figure also clarifies the meanings of $\mu_{\max}$ and K_s.

Sometimes, the growth rate is suppressed in the presence of too much substrate and growth is said to be *substrate inhibited.* In substrate inhibited culture, the specific growth rate attains a maximum value as the substrate concentration is increased and then the growth rate declines, as shown by the dashed line in Fig. 30. Note that in Fig. 30, the $\mu_{\max}$ values are different for the two growth profiles shown. The substrate inhibited growth may be described by the following equation:

$$\mu = \frac{\mu_{\max} S}{K_s + S + \dfrac{S^2}{K_i}}, \tag{7}$$

where K_i is the *inhibition constant.* In other cases, the growth rate may be subject to inhibition by a product of metabolism.

2. Productivity

Productivity of a bioreactor is the quantity of product produced per unit volume in unit time. For a batch bioreactor with the growth profile shown in Fig. 29, the biomass productivity P at any time t is the slope of the straight line

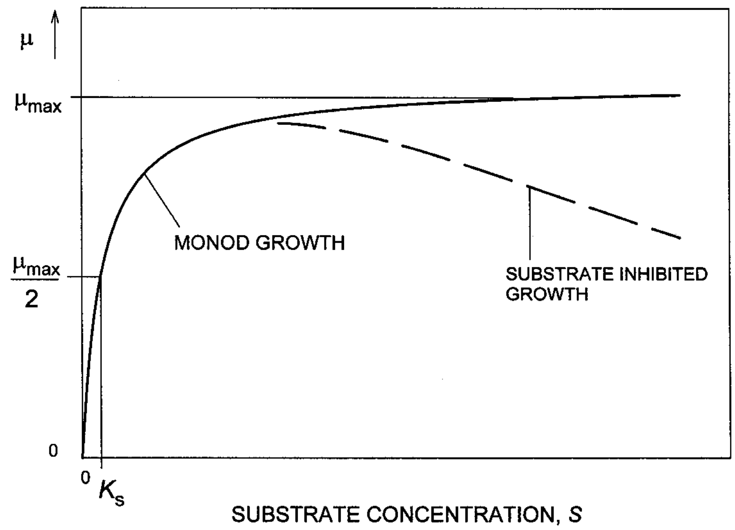


FIGURE 30 Dependence of the specific growth rate μ on the concentration S of the growth limiting substrate.

joining the biomass concentrations at $t = t$ and $t = 0$ in Fig. 31; i.e.,

$$P = \frac{\Delta X}{\Delta t}. \tag{8}$$

This definition of productivity disregards any time invested in preparing the fermenter prior to inoculation of the batch. Lower values of productivity result when the additional preparatory time is taken into account. Often, the product of a fermenter is not the biomass per se, but a compound produced by the cells. Examples of such products are antibiotics and monoclonal antibodies. The kinetics of product formation may mirror those of the cell growth, or they may be quite different. Products known as *secondary metabolites* (i.e., ones that are nonessential to the cell) are often produced after the growth has ceased.

An important operational variable for continuous flow bioreactors is the *dilution rate* D, or the volume flow rate of the feed medium divided by the constant volume of the broth in the reactor. The biomass productivity of a continuous culture is simply the dilution rate D multiplied by the biomass concentration X in the harvest stream. In a continuous flow well-mixed bioreactor, the biomass concentration varies with dilution rate, as shown in Fig. 32. Also, the productivity increases with increasing dilution rate until the dilution rate approaches close to the maximum specific growth rate (Fig. 32). Any further increase in dilution rate causes a sharp decline in productivity and

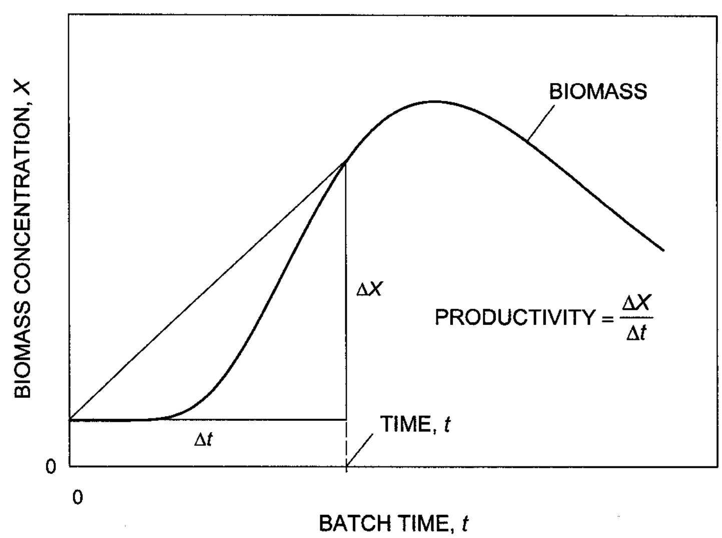


FIGURE 31 Biomass productivity in batch culture.

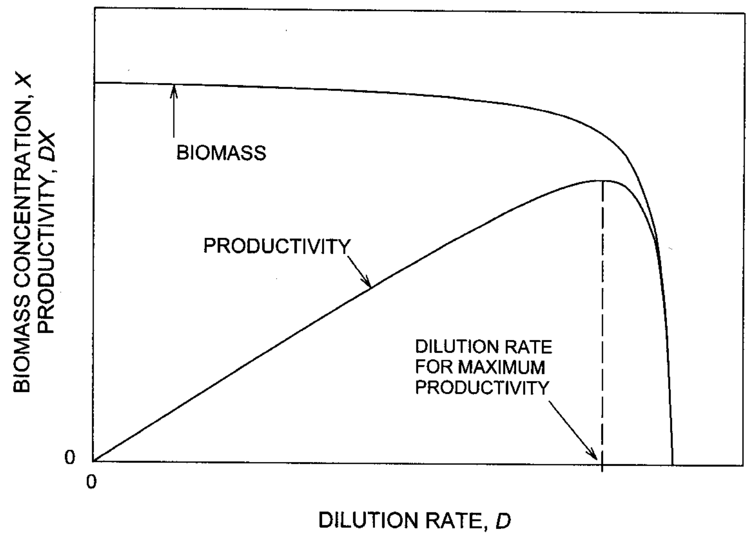


FIGURE 32 Variation of the steady sate biomass concentration and the productivity with dilution rate in a well-mixed continuous flow bioreactor.

washout of cells from the bioreactor. In practice, the dilution rate must remain quite a bit less than the value needed for optimal productivity (Fig. 32), or cells may be washed out because of an inadvertent slight increase in the dilution rate. If viable biomass from the outflow of a continuous flow well-mixed bioreactor is recycled to the reactor, as in Fig. 23b, then the reactor may be operated at a dilution rate greater than the maximum specific growth rate. Such a bioreactor provides a greater biomass productivity compared to one that does not recycle the biomass and the biomass concentration in the reactor outflow stream is also greater.

3. Enzyme Kinetics

Instead of viable cells, a bioreactor may use nonviable cells and isolated enzymes as the biocatalyst. The reaction in such a bioreactor may obey Michaelis-Menten kinetics but other kinetic patterns are also observed. For a reaction that obeys Michaelis-Menten kinetics, the rate of reaction (i.e., the rate of consumption of the substrate, $-dS/dt$) depends on the concentrations of the substrate S and the enzyme e, as follows:

$$-\frac{dS}{dt} = \frac{k_r e S}{K_m + S}, \tag{9}$$

where k_r is the rate constant and K_m is known as the Michaelis-Menten constant. The dynamics of a bioreactor are often analyzed in terms of the *conversion* C_s of the substrate, where C_s is the fraction of the original substrate transformed to a product, i.e.,

$$C_s = \frac{S_o - S}{S_o}. \tag{10}$$

In Eq. (10), S_o is the initial concentration of the substrate in the reactor and S is the concentration at time t.

For Michaelis-Menten kinetics in a well-mixed batch bioreactor, the conversion of the substrate at any time t is governed by the relationship:

$$\frac{k_r E t}{V_L} = S_o C_s - K_m \ln(1 - C_s), \tag{11}$$

where E is the total amount of enzyme in a bioreactor of volume V_L. When the reaction is carried out in a continuous flow well-mixed bioreactor, the expression for the conversion is as follows:

$$\frac{k_r E}{F} = S_o C_s + \frac{K_m C_s}{1 - C_s}, \tag{12}$$

where F is the volume flow rate of the feed. Similarly, in a packed bed bioreactor, the expression for the conversion is the following:

$$\frac{k_r E}{F} = S_o C_s + K_m \ln(1 - C_s). \tag{13}$$

Because F is the volume processed in time t in a continuous flow bioreactor and V_L is the corresponding volume in a batch reactor, a comparison of Eqs. (11) and (13) shows that batch and plug flow (i.e., packed bed) bioreactors containing the same amount of enzyme will achieve equal conversions in a given time. This is a general conclusion, irrespective of the reaction kinetics. A continuos flow packed bed enzyme bioreactor may be advantageous relative to batch reactor, as the unproductive time for batch preparation could be eliminated in the continuous flow unit. However, the batch reactor may have other important advantages such as the ease of pH control in a well-mixed device.

When the substrate concentration S is much greater than K_m, Eqs. (12) and (13) reduce to the same form. In this case, the continuous flow stirred reactor and the plug flow device achieve similar conversion values in a given time. In contrast, when $S \ll K_m$, the reaction rate becomes first order in the substrate concentration (see Eq. (9)), and the plug flow reactor provides higher conversion values in comparison with the well-mixed continuous flow device. In the latter bioreactor, all the enzyme would be exposed to the same low concentration of the substrate which is not useful except when the reaction is inhibited by the substrate.

IV. CONCLUDING REMARKS

A bioreactor is an indispensable part of any bioprocess irrespective of whether the process degrades pollutants or produces substances such as foods, feeds, chemicals and pharmaceuticals, and tissues and organs for use in biomedicine. The variety of bioprocesses is tremendous and many different designs of bioreactors have been developed to meet the different needs. In all cases, the bioreactor must provide the environmental conditions necessary for the culture. The specific demands are often conflicting and achieving optimal performance requires attaining the proper balance among the different requirements. Success of a bioprocess depends critically on good design and operation of the bioreactor.

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BIBLIOGRAPHY

Atkinson, B. (1974). "Biological Reactors," Pion Press, London.

Bailey, J. E., and Ollis, D. F. (1986). "Biochemical Engineering Fundamentals," 2nd Ed., McGraw-Hill, New York.

Chisti, Y. (1989). "Airlift Bioreactors," Elsevier, London.

Chisti, Y. (1999). Solid substrate fermentations, enzyme production, food enrichment. *In* "Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation," (M. C. Flickinger and S. W. Drew, eds.), Vol. 5, pp. 2446–2462, Wiley, New York.

Chisti, Y. (1999). Modern systems of plant cleaning. *In* "Encyclopedia of Food Microbiology" (R. Robinson, C. Batt, and P. Patel, eds.), pp. 1806–1815, Academic Press, London.

Chisti, Y., and Moo-Young, M. (1999). Fermentation technology, bioprocessing, scale-up and manufacture. *In* "Biotechnology: The Science and the Business" (V. Moses, R. E. Cape, and D. G. Springham, eds.), 2nd ed., pp. 177–222, Harwood Academic Publishers, New York.

Deckwer, W. D. (1992). "Bubble Column Reactors," Wiley, New York.

Doran, P. M. (1999). "Design of mixing systems for plant cell suspensions in stirred reactors," *Biotechnol. Prog.* **15,** 319–335.

Moser, A. (1981). "Bioprocess Technology," Springer-Verlag, New York.

Nienow, A. W. (1998). "Hydrodynamics of stirred bioreactors," *Appl. Mech. Rev.* **51,** 3–32.

Tredici, M. R. (1999). Bioreactors, photo. *In* "Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation" (M. C. Flickinger and S. W. Drew, eds.), Vol. 1, pp. 395–419, Wiley, New York.

Van't Riet, K., and Tramper, J. (1991). "Basic Bioreactor Design," Dekker, New York.

Varley, J., and Birch, J. (1999). "Reactor design for large scale suspension animal cell culture," *Cytotechnology* **29,** 177–205.

Willaert, R. G., Baron, G. V., and De Backer, L. (eds.) (1996). "Immobilised Living Cell Systems: Modelling and Experimental Methods," Wiley, Chichester.