Bioreactors: design and operations

Dear students welcome for the lecture series on Industrial Microbiology. Today we are going to discuss another fascinating topic in industrial microbiology, i.e., Bioreactors design and Operations.

The designs of different types of bioreactor can be studied under the following headings,

- **Concept of Bioreactors**
- **Batch bioreactors**
- **Continuous bioreactors**
- **Semi continuous bioreactors**
- **Airlift reactor systems**

I. Concept of Bioreactors

A bioreactor is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Bioreactors arc extensively used for food processing, fermentation, waste treatment etc. On the basis of the agent used, bioreactors are grouped into the following two broad classes: (i) those based on living cells and, (ii) those employing enzymes. But in terms of process requirements, they are of the following types: (i) aerobic, (ii) anaerobic, (iii) solid state, and (iv) immobilized cell bioreactors. All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid.

Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors. But, in general, theoretical explanation usually lags behind technical realization. A bioreactor should provide for the following: (i) agitation (for mixing of cells and medium), (ii) aeration (aerobic fermenters; for O2 supply), (iii) regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (iv) sterilization and maintenance of sterility, and (v) withdrawal of cells/medium (for continuous fermenters). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc. The first truly large-scale aseptic anaerobic fermentation vessels were developed in the rouse of the process developed (during the First World War, 1914-1918) by Weizmann and co-workers of U.K. to produce acetone by a deep liquid fermentation using Clostridium acetobutylicum. For this, large cylindrical vessels of mild steel that permitted sterilization with steam under pressure were constructed, and piping, joints and valves were specially designed to maintain aseptic conditions, which were the major problem; mixing was achieved by the large volumes of gas produced during fermentation. The large-scale aerobic fermentation vessels were first used in Central Europe during 1930s for the production of compressed yeast; these fermenters had large cylindrical tanks in which air was introduced at the base via a network of

perforated pipes. In later modifications, mechanical impellers were used to improve mixing of broth and dispersal of air bubbles.

The goal of an effective bioreactor is to control, contain and positively influence the biological reaction. To accomplish this, the chemical engineer must take into consideration two areas. One is the suitable reactor parameters for the desired biological, chemical and physical (macrokinetic) system. The macrokinetic system includes microbial growth and metabolite production. Microbes can include bacteria, yeast, fungi, and animal, plant, fish and insect cells, as well as other biological materials.

The other area of major importance in bioreactor design involves the bioreaction parameters, including:

• controlled temperature

• Optimum pH

• Sufficient substrate (usually a carbon source), such as sugars, proteins and fats

• Water availability

• Salts for nutrition

• Vitamins

• Oxygen (for aerobic processes)

• Gas evolution and

• Product and byproduct removal.

In addition to controlling these factors, the bioreactor must be designed to both promote formation of the optimal morphology of the organism and to eliminate or reduce contamination by unwanted organisms or mutation of the organism.

Bioreactor/fermentation technologies

Now we will discuss the engineering aspects and applications for a variety of bioreactor/fermentation technologies, including the challenges of each and the advantages and disadvantages of the respective technologies. The various types of bioreactor systems covered here include batch, continuous, semi-continuous, surface/tray, submerged, air-lift loop and trickle-bed setups. As stated before, there are overlapping characteristics in several of the technologies discussed.

II. Batch bioreactors

The majority of bioreactors are batch-wise. The first phase of batch bioreactor is commonly sterilization, after which the sterile culture medium is inoculated with microorganisms that have been cultivated to achieve a specific result.

During this dynamic reaction period, cells, substrates (including the nutrient salts and vitamins) and concentrations of the products vary with time. Proper mixing keeps the differences in composition and temperature at acceptable levels.

To promote aerobic cultivation, the medium is aerated to provide a continuous flow of oxygen. Gaseous by-products formed, such as CO2, are removed, and aeration and gas-removal processes take place semi continuously.

Next, an acid or alkali is added if the pH needs to be controlled. To keep foaming to acceptable levels, antifoaming agents may be added when indicated by a foam sensor.

One of the first types of batch systems is the tray fermentor, used in the early days of commercial aerobic bioreactors for products such as citric acid and penicillin. In this system, the trays are loaded with the culture medium and the organisms, and the air-flow produces the bioreaction, during which exhaust gas is discharged. When the bioreaction is complete, end product is removed from the trays. Because this method is inefficient for producing large commercial quantities, it fell quickly to the wayside with the emergence of submerged tank systems, which are designed to handle significantly higher volumes.

Overall, batch bioreactor systems provide a number of advantages, including:

• Reduced risk of contamination or cell mutation, due to a relatively brief growth period.

• Lower capital investment when compared to continuous processes for the same bioreactor volume.

• More flexibility with varying product/biological systems.

• Higher raw material conversion levels, resulting from a controlled growth period.

The disadvantages include:

• Lower productivity levels due to time for filling, heating, sterilizing, cooling, emptying and cleaning the reactor.

• Increased focus on instrumentation due to frequent sterilization.

• Greater expense incurred in preparing several subcultures for inoculation.

• Higher costs for labor and/or process control for this non-stationary process.

• Larger industrial hygiene risks due to potential contact with pathogenic microorganisms or toxins.

Common applications for batch bioreactors include:

• Products that must be produced with minimal risk of contamination or organism mutation.

• Operations in which only small amounts of product are produced.

• Processes using one reactor to make various products.

• Processes in which batch or semi continuous product separation is adequate.

III. Continuous bioreactors

The defining characteristic of continuous bioreactor is a perpetual feeding process. A culture medium that is either sterile or comprised of microorganisms is continuously fed into the bioreactor to maintain the steady state. Of course, the product is also drawn continuously from the reactor.

The reaction variables and control parameters remain consistent, establishing a time-constant state within the reactor.

The result is continuous productivity and output.

These systems provide a number of advantages, including:

• Increased potential for automating the process.

• Reduced labor expense, due to automation.

• Less non-productive time expended in emptying, filling and sterilizing the reactor.

• Consistent product quality due to invariable operating parameters.

• Decreased toxicity risks to staff, due to automation.

• Reduced stress on instruments due to sterilization.

The disadvantages of continuous bioreactors include:

• Minimal flexibility, since only slight variations in the process are possible (throughput, medium composition, oxygen concentration and temperature).

• Mandatory uniformity of raw material quality is necessary to ensure that the process remains continuous.

• Higher investment costs in control and automation equipment, and increased expenses for continuous sterilization of the medium.

• Greater processing costs with continuous replenishment of non-soluble, solid substrates such as straw.

• Higher risk of contamination and cell mutation, due to the relatively brief cultivation period.

Continuous bioreactor is frequently used for processes with high-volume production; for processes using gas, liquid or soluble solid substrates; and for processes involving microorganisms with high mutation-stability.

Typical end products include vinegar, baker's yeast and treated wastewater.

Continuous vs. Batch fermentors

There are several major advantages to using continuous bioreactors as opposed to the batch mode. First, continuous reactions offer increased opportunities for system investigation and analysis. Because the variables remain unchanged, a benchmark can be determined for the process results, and then the effects of even minor changes to physical or chemical variables can be evaluated. Also, by changing the growth-limiting nutrient, changes in cell composition and metabolic activity can be tracked. The constancy of continuous bioreactor also provides a more accurate picture of kinetic constants, maintenance energy and true growth yields.

Secondly, continuous bioreactor provides a higher degree of control than does batch. Growth rates can be regulated and maintained for extended periods. By varying the dilution rate, biomass concentration can be controlled. Secondary metabolite production can be sustained simultaneously along with growth.

Steady-state continuous bioreactor

In steady-state continuous bioreactor, mixed cultures can be maintained using chemostat cultures — unlike in batch bioreactor, where one organism usually outgrows another. Chemostats are continuous- flow stirred-tank bioreactors (CFSTRs) in an idealized steady-state, i.e., the feed- and outlet-stream compositions and flows are constant, and perfect mixing occurs within the Continuous- flow stirred-tank bioreactors (CFSTR) vessel. In chemostats, the outlet stream composition is considered to be the same as within the bioreactor.

Bioreactors operated as chemostats can be used to enhance the selectivity for thermophiles, osmotolerant strains, or mutant organisms with high growth rates. Also, the medium composition can be optimized for biomass and product formation, using a pulse-and-shift method that injects nutrients directly into the chemostat. As changes are observed, the nutrient is added to the medium supply reservoir and a new steady state is established.

A third advantage is the quality of the product. Because of the steady-state of continuous bioreactor, the results are not only more reliable, but also more easily reproducible. This process also results in higher productivity per unit volume, because time-consuming tasks, such as cleaning and sterilization, are unnecessary. The ability to

automate the process also renders it less labor-intensive, and, therefore, more cost-efficient and less sensitive to the impact of human error.

Along with the strengths of continuous bioreactor, there are inherent disadvantages that may make this process unsuitable for some types of bioreactor. For example, one challenge lies in controlling the production of some non-growth-related products. For this reason, the continuous process often requires feed-batch culturing, and a continuous nutrient supply. Wall growth and cell aggregation can also cause wash-out or prevent optimum steady-state growth.

Another problem is that the original product strain can be lost over time, if it is overtaken by a faster-growing one. The viscosity and heterogeneous nature of the mixture can also make it difficult to maintain filamentous organisms. Long growth periods not only increase the risk of contamination, but also dictate that the bioreactor must be extremely reliable and consistent, incurring a potentially larger initial expenditure in higher-quality equipment.

IV. Semi continuous bioreactors

This hybrid of batch and continuous operations is found in many types of processes. One of the more frequently used is initiating the bioreactor in the batch mode, until the growth-limiting substrate has been consumed. Then, the substrate is fed to the reactor as specified (batch) or is maintained by an extended culture period (continuous). For secondary metabolite production, in which cell growth and product formation often occur in separate phases, the substrate is typically added at a specified rate. Like batch reactors, semi continuous reactors are non-stationary. These systems provide a number of advantages, including:

• Higher yield, resulting from a well-defined cultivation period during which no cells are added or removed.

• Increased opportunity for optimizing environmental conditions of the microorganisms in regard to the phase of growth or production and age of the culture.

• Nearly stationary operation, important with slightly mutating microorganisms and those at risk for contamination.

The disadvantages include:

• Lower productivity levels due to time-consuming procedures for filling, heating, sterilizing, cooling, emptying and cleaning the reactor.

• Greater expenses in labor and/or dynamic process control for the process.

Semicontinuous bioreactors are typically used when continuous methods are not feasible, for example, those in which slight mutation or contamination of the microorganism occurs. Such bioreactors are also used when batch methods do not offer the desired productivity levels.

Submerged bioreactors — stirred tank

The most common type of aerobic bioreactor in use today is the stirred-tank reactor, which may feature a specific internal configuration designed to provide a specific circulation pattern. Ideal for industrial applications, this unit offers manufacturers both low capital and operating costs.

For laboratory experiments with smaller volumes, the mixing vessel is typically made of glass. Stainless steel tank construction is the standard for industrial applications involving larger volumes. The height-to-diameter ratio of the vessel can vary, depending on heat removal requirements.

The operating principles of the stirred-tank bioreactor are relatively simple. The sterile medium and inoculums are introduced into a sterilized tank, and the air supply typically enters at the bottom. For optimal mixing, the tank features not only an agitator system but also baffles, which help prevent a whirlpool effect that could impede proper mixing. In the early stages of the process, warm water may be circulated through the baffles to heat up the system; later, cool water may be circulated inside of them to keep the process from overheating.

The number of baffles typically ranges from four to eight. As the bioreaction progresses, the bubbles produced by the air supply are broken up by the agitator as they travel upward. Many types of agitators are currently used, with the most common one being the four-bladed disk turbine. Newer designs featuring 12 or 18 blades, or concave ones, have also been shown to improve the hydrodynamics. At the top of the tank, exhaust gas is discharged and the product flows back down, where it is drained from the tank.

In a continuous flow stirred-tank reactor, the substrate is continuously fed into the system and the product is continually drawn out and separated, with the producing organism recycled back into the tank for reuse. As with conventional chemical reactors, bioreactors can be placed in series or parallel with controlled recycle streams.

V. Airlift reactor systems

Also known as a tower reactor, an airlift bioreactor can be described as a bubble column containing a draught tube. Many types of airlift bioreactors are currently in use today. Air is typically fed through a sparger ring into the bottom of a central draught tube that controls the circulation of air and the medium. Air flows up the tube, forming bubbles, and exhaust gas disengages at the top of the column.

The degassed liquid then flows downward and the product is drained from the tank. The tube can be designed to serve as an internal heat exchanger, or a heat exchanger can be added to an internal circulation loop.

Airlift systems provide some advantages vs. more conventional bioreactors, such as the standard fermenter:

• Simple design with no moving parts or agitator shaft seals, for less maintenance, less risk of defects and easier sterilization.

• Lower shear rate, for greater flexibility — the system can be used for growing both plant and animal cells.

• Efficient gas-phase disengagement.

• Large, specific interfacial contact-area with low energy input.

• Well-controlled flow and efficient mixing.

• Well-defined residence time for all phases.

• Increased mass-transfer due to enhanced oxygen solubility achieved in large tanks with greater pressures.

• Large-volume tanks possible, increasing the output.

• Greater heat-removal vs. conventional stirred tanks.

The main disadvantages are:

• Higher initial capital investments due to large scale processes.

• Greater air and higher pressures needed, particularly for large-scale operation.

• Low friction with an optimal hydraulic diameter for the riser and down comer.

• Lower efficiency of gas compression.

• Inherently impossible to maintain consistent levels of substrate, nutrients and oxygen with the organisms circulating through the bioreactor and conditions changing.

• Inefficient gas/liquid separation when foaming occurs.

However, these disadvantages can and must be minimized in designing airlift systems. For example, if only one location serves as the feed source, the organism would experience continuous cycles of high growth, followed by starvation, resulting in the production of undesirable by-products, low yields and high mortality rates. A design with multiple feed points eliminates this risk, especially for large-scale operations.

The same risks are inherent in a single entry point for oxygen, which must be delivered at various places within the vessel, with the majority of the air entering at the bottom to circulate the fluid through the reactor.

Airlift external-loop reactors

Another type of airlift system is the airlift external-loop reactor system, used primarily for batch operation. A variation of the airlift system, the airlift external-loop reactor (AELR) uses induced circulation to direct air and liquid throughout the vessel. This

system consists of a riser and an external down comer, which are connected at the bottom and the top, respectively. As the injected air at the bottom of the riser creates gas bubbles that begin to rise through the main tank, exhaust gas disengages at the top and the resulting heavier solution descends through the down comer.

The airlift external-loop reactor (AELR) has some advantages over standard airlifts:

• Effective heat-transfer and efficient temperature control.

• Low friction with an optimal hydraulic diameter for both the riser and down comer.

• Well-defined residence time in the individual section of the airlift external-loop reactor (AELR).

• Increased opportunity for measurement and control in the riser and the down comer.

• Independent control of the gas input-rate and liquid velocity by a throttling device between riser and down comer.

Conclusion

Bioreactors will be integral to the development of many new high-value products and the replacement of existing chemical-based commodity processes. The proper selection and design of the bioreactor will determine the optimal commercial bioprocess and the corresponding capital investment.

The bioreactor should not be regarded as an isolated unit, but as part of an integrated unit operation with both upstream (preparation) and downstream (separations) unit operations.

Thank you