Science Education Collection

Batch and Continuous Bioreactors

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Abstract

Bioreactors are used to grow organisms in large volumes, thereby enabling the production of mass quantities of the target product. These reactors can be batch reactors, which contain all of the components needed for cell growth, or continuous reactors, which have inlet and outlet ports allowing for the addition of fresh growth media and the removal of cell waste.

This video presents batch and continuous reactors, and demonstrates the use of bioreactors to grow bacteria in the laboratory. Finally, this video considers how these reactors are used in the bioengineering field to produce products such as protein therapeutics or even beer.

Transcript

Bioreactors, also known as fermenters, are cell culture systems that provide suitable growth environments. Two types of bioreactors are commonly used in cell culture, batch and continuous stirred tank bioreactors. A batch reactor is a closed vessel system containing all of the components needed for cell culture. Whereas a continuous stirred tank reactor is an open system where components are actively flowing in and out, enabling the removal of waste and the replenishment of nutrients. To maximize cell growth and cell density, multiple reactor parameters can be controlled for both systems, including temperature, pressure, dissolved oxygen, and pH. This video will introduce the fundamental principles behind batch and continuous reactors and demonstrate a procedure detailing their use in the laboratory.

The simplest type of bioreactor is the batch reactor, which is a closed system containing cell culture media and a population of cells. As the cells grow, they consume the nutrients and excrete waste, which accumulates in the vessel. Continuous stirred tank reactors are also used, which are open systems where nutrients continuously flow in, and cells and cell waste flow out. This enables the control of cell density via the removal of waste and the replenishment of nutrients. Prior to use of either system, the components are sterilized, usually using steam, in order to mitigate contamination. When the reactor is fully assembled, sterile culture media is added to the main vessel and then inoculated with a starter culture. During use, mixing and oxygenation are controlled with the use of impellers to maintain a homogenous solution where cells are supplied with sufficient amounts of nutrients and oxygen needed for growth. Additionally, fermenter tank systems are often equipped with probes to measure conditions such as temperature, dissolved oxygen, and pH to ensure optimal conditions for growth. During batch growth, the cell population undergoes classic growth phases. First, the cells enter lag phase, where cells grow slowly as they adapt to their environment. Next, cells enter log phase and divide at an exponential rate until the nutrients are exhausted or toxic byproducts reach a critical level. Eventually, growth slows and cells reach the stationary phase which provides an opportunity to harvest the product of interest from the culture. The product is either secreted by the cells and collected directly from the culture broth, or the product remains in the cell and must be removed via cell lysis. After harvesting, the reactor is prepared for a new cell culture batch by cleaning and sterilization. Continuous stirred tank reactors exhibit similar growth patterns initially, and reach a constant cell density at steady state operation. However, this cell density is dependent on the dilution rate, which is the feed and effluent rate divided by the reactor volume. Thus, as the dilution rate approaches one, the cell density decreases. When considering bioreactor configurations, it is helpful to keep in mind that batch reactors are often used because of their simplicity and low cost, although cell density is limited. Continuous reactors are able to increase cell density. However, at high cell densities aggregation can occur, preventing optimum growth. Additionally, longer periods of fermentation can increase the risk of culture contamination.

Now that we have introduced batch and continuous reactors, let's examine a procedure for both, starting with the batch reactor. Prior to batch reactor use, cell media is added to a clean reaction vessel. Once filled, the vessel components are rinsed with deionized water and assembled. The components include the head plate, the harvest pipe for culture sampling, an impeller for maintaining culture uniformity and media oxygenation, and gas sparger to provide gas infusion to the culture. Assembly continues with the installation of an oxygenation probe to measure the dissolved oxygen content in the vessel, feed lines for adding acid or base to control pH, and a calibrated pH probe. When all components are installed, the assembled reactor is sterilized using an autoclave. The vessel is then installed in the fermenter base, where both the pH probe and oxygen probe are connected to the computer. Additionally, the gas sparger is connected to a gas rotameter, which measures gas flow rate. At this time, the impeller is turned on and the gas flow, temperature, and vessel agitation are adjusted until the desired parameters are achieved. The inoculation port is then sterilized with alcohol and the starter culture dispensed into the reactor vessel. During operation, cell culture samples are drawn at selected time points to construct a growth curve based upon cell density measurements. When the cell density reaches the desired level, the culture is stopped and the cells are harvested from the vessel through a series of filtration steps. The remaining vessel contents are disposed of using bleach or other anti-microbial contents.

Similar to batch reactors, continuous stirred tank reactors, sometimes called chemostats, require their individual components to be rinsed thoroughly with deionized water before assembly. Once cleaned, the stirrer assembly and drive shaft components are fitted together, and the reactor vessel filled with deionized water to improve sterilization during autoclaving. Dissolved oxygen, pH, and temperature probes are calibrated, then subsequently installed. With the vessel fully assembled, the reactor is sterilized using an autoclave. After sterilization, the vessel is placed in a heat jacket to control its temperature, and the calibrated probes are connected. Then, the media carboy and air supply with a sterile filter are connected before turning the air flow on. The vessel is primed with sterile media by unclamping the carboy tubing, and then running the basic fermenter program. As the media flows into the active reactor, the inoculation port is sterilized with alcohol before one milliliter of starter culture is added to the vessel. During operation, culture samples are taken at selected time points to measure cell density. This data is then used to construct a growth curve. Optimal growth is achieved when the culture reaches steady state, meaning that the cell density remains constant.

Now that you have learned about batch and continuous stirred tank reactors, let's look at some practical applications of this technology. Beer brewing is typically performed by growing yeast in a batch reactor system. A set of basic ingredients consisting of purified water, malted barley,

hops, and yeast are added to a reactor. The malted barley stimulates the fermentation process by acting as a sugar-rich food source for the yeast, which, when consumed, results in the production of alcohol as a waste product. Once the mixture has reached its target alcohol content, or fermented for the prescribed amount of time, it can be filtered, packaged, and left to age to induce carbonation before the final product is distributed. Standard reactors can also be customized for specialized purposes. For example, specialized reactors can be used to increase the cell viability of tissue scaffolds through enhanced cell and nutrient distribution by constant mixing. Simultaneously, mechanical stimulation can be applied to the scaffold to encourage extra-cellular matrix production and actively direct cell growth and differentiation. The resulting scaffolds have improved physiological and mechanical properties, making them appealing for implantation.

You've just watched Jove's video on batch and continuous stirred reactors. You should now understand how batch and continuous stirred tank bioreactors work, and how these systems can be applied in the bioengineering field. Thanks for watching.