PharSol BLOG

https://pharsol.com/knowledge-hub/blog/bioreactor-operation-guide

## Introduction

In industry microbial fermentation is used to produce diverse products, from biopharmaceuticals, food ingredients, biofuels and more (we've written a blog post on that topic already, so make sure to check that one out). In order to perform such cost-effective processes on an industrial scale, various factors must be considered, and an optimal mode of operation has to be selected.

Bioreactors have generally 3 modes of operation for cell cultivation, which are determined by the flow rates in and out of the system. The three popular modes of operation are batch, fed-batch and continuous operations.

As with all processes, there are advantages and disadvantages attributed to every mode of operation. Main advantages, as well as disadvantages, are listed in each chapter below the description of each mode of operation.

###### A diagram of different bioreactor modes of operation

## Mode of operation: Batch

Batch operation is defined as a closed process, meaning there is no inflow and outflow of the reactor during the complete run. It is the most simple mode of bioreactor operation. In this mode, all the components are added at the beginning of the fermentation run in a fixed bioreactor volume.  There is no addition of substrate or medium during the run of the bioprocess, with the exception of adding gas for aeration and base to control the pH. Therefore in the beginning high concentrations of the substrate need to be supplied to prevent nutrient limitation, which can limit final biomass and product concentration. At the end of the run, the whole batch is taken for downstream processing and the reactor is then prepared for the new fermentation process.

During the run, conditions become variable, as the substrate consumption increases and product concentration increases due to growing cell concentration. At first, cells are in the exponential phase and have unlimited growth. The cell density grows until the substrate is depleted. Once substrate becomes the limiting factor cell death occurs, leading to zero growth rate at the end.

###### Graph 1: Cell growth in a batch operation mode bioreactor system, in relation to substrate consumption (S) and product production (P)

Due to variable conditions during the run product quality can change. The process outcome is determined by the starting conditions.

### Advantages

* Operational simplicity, robust
* GMP friendly
* Cells have growth independent productivity – meaning, they can produce as long as they are viable.

### Disadvantages

* Low biomass and product concentration.
* A lot of nutrients invested in the growth
* Short process and more down time.
* Overflow metabolism – cells produce a lot of lactate and ammonia during their growth, which can become toxic to the cell culture and can lead to accelerated cell death.
* Varying conditions may affect product quality.
* Initial conditions are important – no deviation in the complex media (hard to do).
* Scale of the process is limited.

## Mode of operation: Fed-Batch (FB)

Fed-batch mode of operation is a modified version of a batch fermentation process and is most commonly used in the industry. Initially, the fed-batch mode of operation starts as a batch phase, where substrate decreases and cells grow exponentially. Before the substrate becomes limiting, feed (concentrated amino acids and glucose) is added at a rate of consumption throughout the remaining fermentation duration. The rate of feed addition determines growth rate. By controlling the rate of feed addition, high cell densities can be reached. Conditions in the bioreactor are kept constant, with the exception of the growing biomass, product and waste product concentrations. Cells are growing and producing until the maximum density of the reactor is reached. By using low substrate concentrations in fed-batch mode, waste metabolism (lactate production) in the culture can be minimized or avoided.

In theory, the fed-batch process would look like Graph 2.

###### Graph 2: Theoretical visualisation of cell growth, product production and substrate consumption in a fed-batch system.

However, in practice, with the addition of the substrate, the process resembles graph 3.

###### Graph 3: Actual visualisation of cell growth, product production and substrate consumption in a fed-batch system.

### Advantages

* Can reach much higher cell densities and product than in batch.
* Less downtime due to a longer process, leading to less overflow metabolism.
* More constant conditions.
* Down-scalable process – allows for fast process development.

### Disadvantages

* More complex than batch, due to the addition of the feed.
* More prone to errors.
* No removal of waste products and/or cell debris.
* Scale is determined by the risk of losing the run and final amount of product.

## Mode of operation: Continuous (Chemostat)

Continuous mode of operation is an open process, where both inflow and outflow are constant. Fresh medium is continuously supplied and old culture medium together with the cells is continuously taken out at the same rate. This results in cells continuously receiving fresh medium with nutrients increasing their productivity, while products and waste products together with cells are continuously removed for further processing, which increases the viability of the culture. Therefore, in contrast to fed-batch fermentation, the maximum working volume of the vessel is not limited by the amount of fresh medium or feed solution which can be added to the culture during the process run. At a certain moment, the system reaches a steady state.

###### Graph 4: Cell growth in a continuous mode of operation, in relation to substrate consumption and product production.

### Advantages

* Production optimization – faster production times
* Automation
* Less downtime.
* You are able to control the growth rate (determined by the rate you refresh the medium) – manipulate the balance between product formation and biomass.
* Removal of waste metabolites and less waste metabolism, due to substrate concentration being continuously low.
* Constant conditions.

### Disadvantages

* The system is more complex – a higher probability of error.
* More risk - if the run is continuous and something goes wrong a complete run is lost.
* Low biomass and product.
* The stability of the culture is compromised, meaning each time a cell divides a mutation can happen with a potential loss of productivity.
* Thus the stability of the cell line overtime must be demonstrated beforehand.
* Medium use is inefficient.

## Improvement to continuous mode: Perfusion

Perfusion is a continuous culturing method with implemented cell retention. Fresh medium is continuously added and old medium is removed through a filter. The filter blocks the passage of cells, allowing only the harvested medium to pass through. This results in higher cell concentration and product yield. However, cell death still occurs to some degree and some cell debris stays inside the bioreactor. To avoid lower cell viability due to this factor there is bleed flow, where some of the cells are taken out of the bioreactor (lesser degree than in chemostat). Cell retention must be performed in a sterile way. There are many cell retention options, such as settlers, centrifuges, internal and external filters, and more are in development.

Perfusion culture ideally looks like this:

Substrate concentration is maintained at a certain level, reaching a steady state, where all conditions are constant. Due to retention of the cells cell concentrations are very high, even close to tissue density.

However, in practice cell concentration does not remain stable and product concentration does not reach the steady state. This is related to the complexity of the media and metabolism of the cell.

### Advantages

* Perfusion systems can reach very high product concentration and cell viability.
* The growth rate is controlled by medium and nutrients by retention – independent control.
* Clear separation of growth and production phase.
* Constant conditions and less downtime.
* In-line cell separation – easier for downstream processing.
* Higher volumetric productivity – more product in a smaller reactor.

### Disadvantages

* cell retention is difficult – complex.
* Cell death and debris foul probes and filters.

## Sources

* Bioprocess Engineering Principles (Second Edition): Reactor engineering  
  (<https://www.sciencedirect.com/science/article/pii/B9780122208515000149>)
* A beginner’s guide to Bioprocess modes - Batch, Fed Batch and Continuous Fermentation  
  (<https://bit.ly/3zkhtYI>)
* Fermentation modes  
  (<https://bioprocessingexplained.home.blog/2019/09/02/fermentation-modes/>)
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  (<https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118869703.ch2>)
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