

## 2.4 TYPES OF MICROBIAL CULTURE

Microbial culture processes can be carried out in different ways. There are three models of fermentation used in industrial applications: batch, continuous and fed batch fermentations.

### 2.4.1 BATCH FERMENTATION

A batch fermentation system is a closed system. At time  $t=0$ , the sterilized nutrient solution in the fermenter is inoculated with microorganisms and incubation is allowed to proceed at a suitable temperature and gaseous environment for a suitable period of time. In the course of the entire fermentation nothing is added, except oxygen (in case of aerobic microorganisms), an antifoam agent, acid or base to control pH. The composition of the medium, the biomass concentration and the metabolite concentration generally change constantly as a result of metabolism of the cells. After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, six typical phases of growth are observed (Fig. 2.2).

Growth is a result of consumption of nutrients. The initial **lag phase** is a time of no apparent growth but actual biochemical analyses show metabolic turnover, indicating that cells are in the process of adapting to the environmental conditions and that new growth will eventually begin. There is then a **transient acceleration phase** as the inoculum begins to grow, which is quickly followed by an **exponential phase**. In the exponential phase, microbial growth proceeds at the maximum possible rate for that organism with nutrients in excess, ideal environmental parameters and growth inhibitors absent. However, in batch cultivations exponential growth is of limited duration and as nutrient conditions change, growth rate decreases, entering the **deceleration phase**, to be followed by the **stationary phase**, when overall growth can no longer be obtained owing to nutrient exhaustion. The final phase of the cycle is the **death phase** when growth rate has ceased. Most biotechnological batch processes are stopped before this stage because of decreasing metabolism and cell lysis. Typical microbial cultures in the laboratory (in a flask) are batch cultures.

Batch culture systems provide a number of advantages:

- 1 Reduced risk of contamination or cell mutation as the growth period is short.
- 2 Lower capital investment when compared to continuous processes for the same bioreactor volume.
- 3 More flexibility with varying product/biological systems.
- 4 Higher raw material conversion levels, resulting from a controlled growth period.

The disadvantages include:

- 1 Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
- 2 Increased focus on instrumentation due to frequent sterilization.
- 3 Greater expense incurred in preparing several subsultures for inoculation.
- 4 Higher costs for labour and/or process control for this non-stationary procedure.
- 5 Larger industrial hygiene risks due to potential contact with pathogenic microorganisms or toxins.

Common applications for batch cultures include:

- 1 Products that must be produced with minimal risk of contamination or organism mutation.
- 2 Operations in which only small amounts of product are produced.
- 3 Processes using one reactor to make various products.
- 4 Processes in which batch or semi-continuous product separation is adequate.

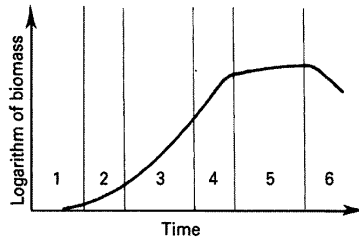


Fig. 2.2: Growth characteristics in a batch culture of a microorganism, 1: lag phase, 2: transient acceleration, 3: exponential phase, 4: deceleration phase, 5: stationary phase, 6: death phase (Smith, 2004).

#### 2.4.2 CONTINUOUS FERMENTATION

In continuous fermentation an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system (Fig. 2.3). In a homogeneously mixed bioreactor, we can have a chemostat or a turbidostat. In the chemostat, in the steady state adjusting the concentration of one substrate controls cell growth. In the turbidostat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted. In the chemostat, constant chemical environment is maintained, while in a turbidostat constant cell concentration is maintained.

In a chemostat the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from the reservoir. The volume of fluid in the growth chamber is maintained at a constant level by some sort of overflow drain. Fresh medium is allowed to enter the growth chamber at a rate that limits the growth of the bacteria. The rate of addition of fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances and the accumulation of excess cells in the culture which are the parameters that initiate the stationary phase of the growth cycle.

There are several major advantages of using continuous cultures as opposed to batch cultures:

- 1 Continuous reactions offer increased opportunities for system investigation and analysis. As 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. By changing the growth-limiting nutrient, changes in cell composition and metabolic activity

can be tracked. The constancy of the continuous process also provides a more accurate picture of kinetic constants, maintenance energy and true growth yields.

- 2 Continuous culture provides a higher degree of control than a batch culture. 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. In steady state continuous culture, mixed cultures can be maintained using chemostat cultures – unlike in a batch process where one organism usually outgrows another.
- 3 Bioreactors operated as chemostats can be used to enhance 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.
- 4 Because of the steady state of continuous culture, the results are not only more reliable but also more consistent leading to a better quality product.
- 5 It also results in higher productivity per unit volume, as time consuming tasks, such as cleaning and sterilization are unnecessary.
- 6 The ability to automate the process makes it more cost-efficient and less sensitive to the impact of human error.

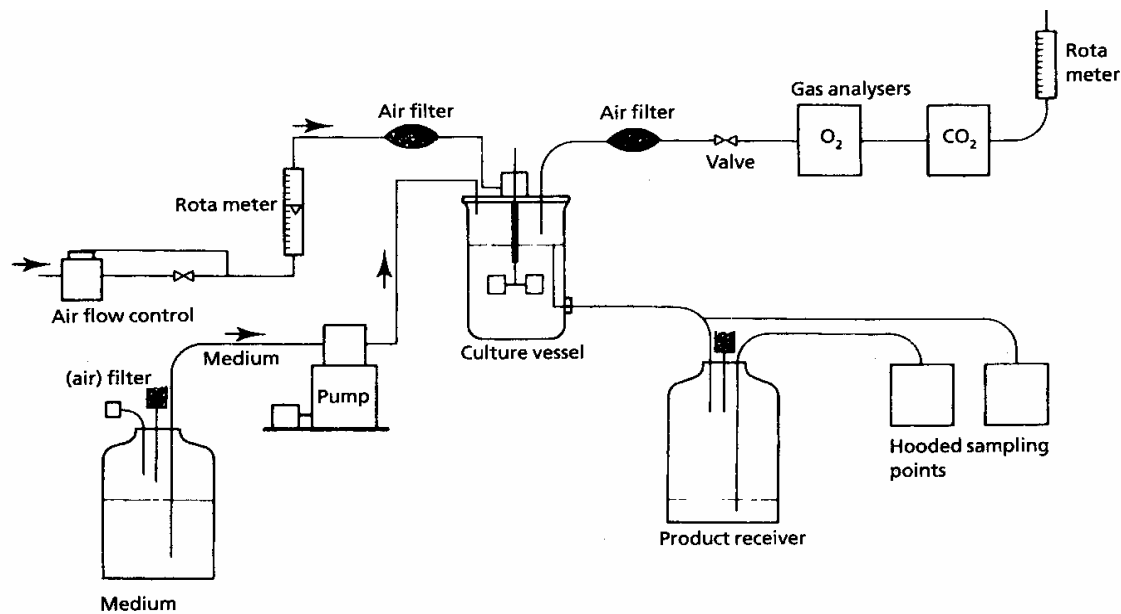


Fig. 2.3: A simple laboratory fermenter operating on a continuous cultivation basis (Smith, 2004).

Disadvantages include:

- 1 The control of the production of some non-growth related products is not easy. For this reason, the continuous process often requires feed-batch culturing and a continuous nutrient supply.
- 2 Wall growth and cell aggregation can also cause wash-out or prevent optimum steady-state growth.
- 3 The original product strain could be lost over time if a faster growing one overtakes it.
- 4 The viscosity and heterogenous nature of the mixture can also make it difficult to maintain filamentous organisms.
- 5 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.

#### 2.4.3 FED-BATCH FERMENTATION

The fed batch method is characterized by the addition of small concentrations at the beginning of the fermentation and these substances continue to be added in small doses during the fermentation process. Despite the apparent similarity between the fed batch reactor model and the continuous culture model, they are very different. Whereas the chemostat process (continuous culture) for biomass accumulation is composed of a growth and removal process, the fed batch procedure is composed of a growth and dilution process.

The concept of steady state cannot be easily applied to a fed batch reactor. It is significantly more difficult to maintain a specific growth rate in a fed batch system than in continuous culture. As cells are not removed during the fermentation, fed batch cultures are well suited for the production of compounds produced during very slow or zero growth. Unlike a continuous culture, the feed does not need to contain all the nutrients needed to sustain growth. The feed may contain only a nitrogen source or a metabolic precursor.

Contamination and/or mutation will not have the same dramatic effect on a fed batch fermenter. A fed batch fermenter can be operated in a variety of ways, e.g., the reactor can be operated in the following sequence: Batch => Fed batch = > Batch. The feed can also be manipulated to maximize product formation. During fermentation, the feed composition and feed flow rate can be adjusted to match the physiological state of the cells. Fed batch reactors can maintain low nutrient and substrate concentrations and are thus well suited for producing product or cells when the substrate is inhibitory by allowing the maintenance of low levels of substrate so that cells are not inhibited. They are very useful for the production of vinegar and amylase.

Fed batch fermentations are also useful when the product or biomass yield is highest at low substrate concentrations as in the case of mammalian cell systems for recombinant protein, baker's yeast products and antibiotic production. Another suitable application is when the product formation is dependent on a specific nutrient composition, e.g., specific carbon to nitrogen ratio.

Advantages of fed batch systems:

- 1 Higher yield, resulting from a well-defined cultivation period during which no cells are added or removed.
- 2 Increased opportunity for optimizing environmental conditions of the microorganisms in regard to the phase of growth or production and age of the culture.
- 3 Nearly stationary operation, important with slightly mutating microorganisms and those at risk for contamination.

Disadvantages include:

- 1 Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
- 2 Higher costs in labour and/or dynamic process control for the process.