### PRINCIPLES OF FERMENTATION

Fermentation is the process that produces acids and alcohol from carbohydrate sources under anaerobic conditions. It is a metabolic process that produces chemical changes in organic substrates through the action of microorganism and/or their enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. In the context of food production, it may more broadly refer to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage. Humans have used fermentation to produce foodstuffs and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid found in such sour foods as yogurt or pickled cucumbers, as well as for producing alcoholic beverages such as wine and beer.

### FERMENTOR AND BIOREACTOR

A fermentor is simply an optimal environment for bacteria and / or fungi to grow in, and the cultivation of said organisms will yield a desirable substance..

A bioreactor is a vessel in which a biochemical process is carried out which involves organisms or biochemically active substances derived from such organisms (e.g. enzymes). Bioreactor is a system used for the growth and maintenance of a population of mammalian or insect cells whereas.

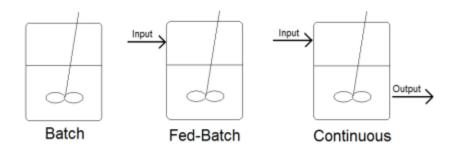
In summary; the main difference between bioreactor and fermentor is the type of biochemical reaction that takes place inside sealed vessels. A bioreactor performs all kinds of biochemical reactions i.e. bioprocesses, but a fermentor only performs fermentation.

# **BIOPROCESSING**

Bioprocessing is the use of biological materials (organisms, cells, organelles, enzymes) to carry out a process for commercial, medical or scientific reasons. Bioprocess operations should ideally manufacture new products and destroy harmful wastes. Use of microorganisms to transfer biological material for production of fermented foods has been an essential part of many foods, chemical and pharmaceutical industries.

### **METHODS of FERMENTATION**

A fermentation system is usually operated in one of the following modes: batch, fed batch, or continuous fermentation. The choice of the fermentation mode is dependent on the relation of consumption of substrate to biomass and products.



### -BATCH FERMENTATIONS

In batch fermentation, microorganisms are inoculated to a fixed volume of medium in a fermentor. Fermentation proceeds, the nutrients are gradually consumed and byproducts accumulate. After the proper time the contents of the fermenter, are removed at the end of the run for further processing. The fermenter is cleaned and the process is repeated. Therefore the culture environment is continuously changing.

The growth curve is usually divided into distinct phases. During the initial lag phase, growth is slow, as the organism needs to adapt to the new environment. During the exponential growth phase, the microbes divide at a constant rate. When nutrients are getting depleted and by-products accumulate, growth slows down, and the culture enters the stationary growth phase. At this point, bioprocess engineers usually harvest the culture. If the culture continues, it would finally enter the death phase, which is characterized by a decrease in the viable cell density.

The advantages of batch processing are ease of operation and low risk of contamination. Disadvantages are the comparatively low cell densities which can be achieved and the relatively long downtime between batches, due to cleaning, vessel setup, and sterilization.

Batch fermentation is a convenient starting point for beginners in this field, and is often used to optimize conditions in the early stages of experimental design.

### -CONTINUOUS FERMENTATION

In continuous fermentation, fresh medium is continuously added to the fermentor, while used medium and cells are harvested at the same time. Consumed nutrients are replaced and toxic metabolites are removed from the culture. When addition and removal are at the same rate, the culture volume stays constant. Therefore, in contrast to fed-batch fermentation, the maximum working volume of the vessel does not limit the amount of fresh medium or feed solution which can be added to the culture in the course of the process.

Growth of microorganisms during batch fermentation confirms to the characteristic growth curve, with a lag phase followed by a logarithmic phase. This, in turn, is terminated by progressive decrements in the rate of growth until the stationary phase is reached. This is because of limitation of one or more of the essential nutrients. In continuous fermentation, the substrate is added to the fermenter continuously at a fixed rate. This maintains the organisms in the logarithmic growth phase. The fermentation products are taken out continuously. The design and arrangements for continuous fermentation are somewhat complex.

The rate of medium exchange can be optimized to reach a steady state.

**Steady state:** the cellular growth rate and environmental conditions, like the concentrations of metabolites, stay constant. Cultures in steady-state can last for days, weeks or even months, thus greatly reducing the downtime and making the process more economically competitive. Due to the long cultivation, sterility maintenance can be challenging, and downstream processing is complicated.

A **chemostat** (chemically *static*) is a <u>bioreactor</u> to which fresh medium is continuously added, while culture liquid containing left over nutrients, metabolic end products and microorganisms are continuously removed at the same rate to keep the culture volume

constant. By changing the rate with which medium is added to the bioreactor the <u>specific</u> growth rate of the <u>microorganism</u> can be easily controlled within limits.

A **turbidostat** is similar to a <u>chemostat</u> which has feedback between the <u>turbidity</u> of the culture vessel and the dilution rate.

#### -FED BATCH FERMENTATION

Fed-batch fermentation is a modified version of batch fermentation. Microorganisms are inoculated and grown under batch regime for a certain amount of time, then nutrients are added to the fermenter in increments throughout the remaining duration of fermentation to feed them. The entire culture suspension is removed at the end of each run. The start of feeding is normally determined by substrate limitation in the broth, and the time profile of feeding should be designed in a way that the substrate remains non-excessive while microbial growth is fully supported. Because of the addition of fresh nutrients, extensive biomass accumulation normally occurs in the exponential growth phase. Therefore, fed-batch fermentation is very useful for bioprocesses aiming for high biomass density or high product yield when the desired product is positively correlated with microbial growth. Also, because the substrate is not overfed during the process, by-product accumulation is limited.

### TYPES OF FERMENTATION

Fermentation systems may be **submerged** (**liquid**) or **solid state** (**surface**). Most fermentors used in industry are of the submerged type, because the submerged fermentor saves space and is more amenable to engineering control and design.

# SUBMERGED LIQUID FERMENTATION

Submerged fermentation involves submersion of the microorganism in an aqueous (liquid) medium containing all the nutrients needed for growth.

Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic metres. The fermentation media sterilises nutrients based on renewable raw materials like maize, sugars and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimize the fermentation process.

# **SURFACE or SOLID-STATE FERMENTATION (SSF)**

SSF is defined as fermentation involving solids in the absence of free water, although the substrate must possess sufficient moisture to support microbial growth and metabolism.

In the surface techniques, the microorganisms are cultivated on the surface of a liquid or solid substrate. These techniques are very complicated and rarely used in industry. Surface fermentation is easy to control and to implement. It needs no aeration or agitation of the fermentation broth, so it needs no instrumentation for aeration and agitation. However, surface fermentation has the following disadvantages: Building investment costs are high. Personnel expenses are high in developed industrial countries with extremely high wages. Fermentation time is long and therefore productivity is low.