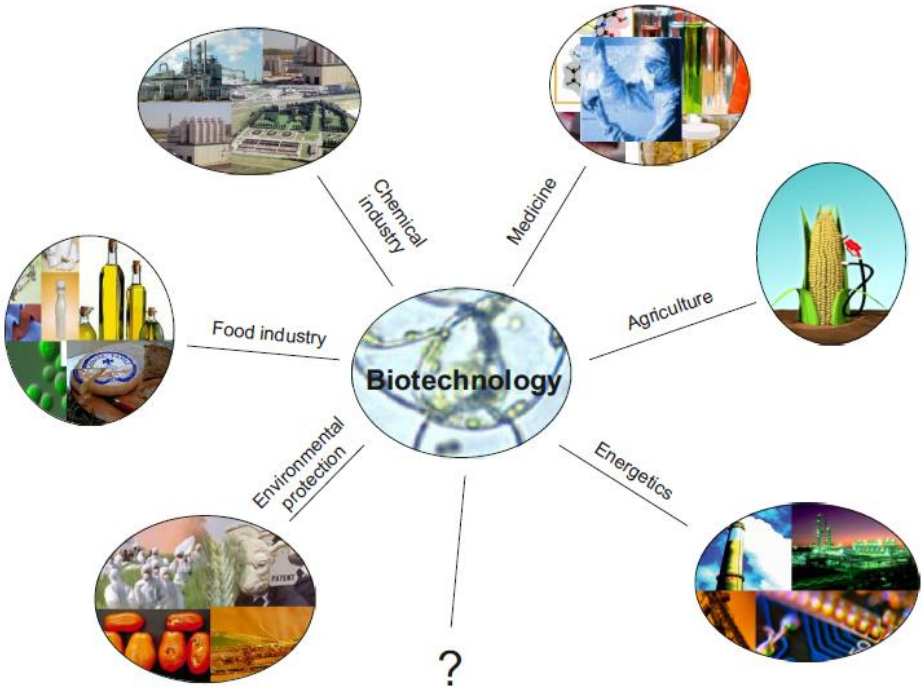
What is biotechnology?

Biotechnology is a field that deals with obtaining products, substances and processes necessary for humans by using microorganisms. Origins of biotechnology are connected with wine and cheese production already in olden times. Of course, at that time such terminology was not used. Today, it is not so easy to understand, where the limits of the biotechnology's applicability are, as it is combined with other scientific and technical fields such as molecular biology, genetics and gene engineering, biochemistry, chemical technology, etc.

Biotechnology has pushed into our everyday life more than we usually imagine. It has offered applications for different fields:

1. For food industry requirements - citric acid, amino acid and other food additives;
2. For agriculture - plant protection agents, modified food;
3. For medicine - antibiotics, interferon, vitamins, vaccines and other preparations;
4. For environment protection - contamination degrading substances and processes;
5. In energetics - biogas, ethanol and other energy sources;
6. For the chemical industry - ethylene, acetone, buthanol and other substances.



And these are not the only applications. There are studies and developments which make it possible to augment more and more complicated live cells. Biotechnology continues to develop, and nobody will take the risk of predicting its development

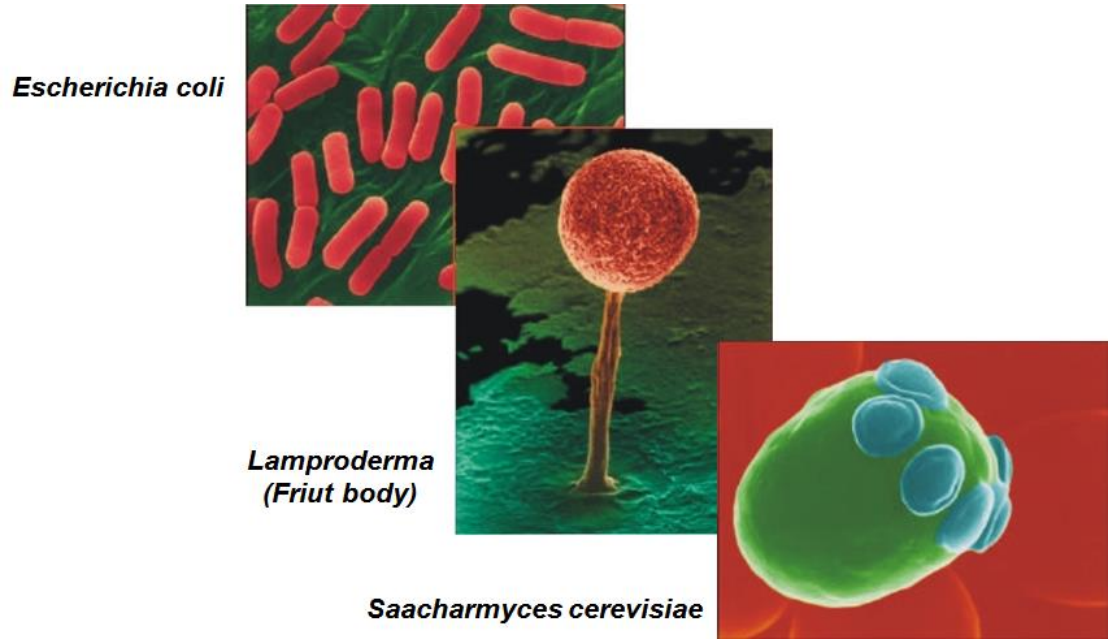
potentialities. Its development rates have already reached such level that makes the society to control its progress.

Usually for biotechnological process there are 3 major stages in order to reach the necessary goal:

1. Preparation of nutrient media for the cultivated microorganism and the cultivation process;
2. The course of the microorganism reproduction process in bioreactors (also called fermenters) or in other equipment for cultivation;
3. The final product or substance obtaining from the cultivated medium. This stage includes such operations as separation, purification and other technologies that are connected with obtaining the commodity form.

What is the bioreactor necessary for?

Bioreactor is a device, usually a vessel, that provides the necessary conditions for microorganisms reproduction. The size of microorganisms depends on their type. However, in any case, we are dealing with several μm. When microorganisms reproduce their number can reach even 1 million cells/ml. Here are some electron microscopy images of microorganisms:



Microorganisms grow in a nutrient medium, from which the energy required for growth is taken. In addition as the nutrient element can be used oxygen that is supplied with the help of compressed air. To provide a successful microorganism

growth, it is necessary to precisely observe the environmental conditions, because microorganisms are very sensitive. Therefore, in the bioreactor there should be provided a very high comfort level. The temperature cannot vary by more than 1°C to one or the other side. The environment should be as acidic as it is known to be the best for the given microorganisms. The more microorganisms have grown, the more oxygen should be given to them. Do not forget to check if all of the necessary nutrient components are retained in medium, otherwise, because of their sensitivity they can either die or fail to do the work they had been entitled to do, i.e. starts synthesis of some different and undesirable products. But - do not exaggerate, for example if too much oxygen is given, they can "choke" and it is difficult to predict the sequences. In addition, it should be kept in mind that this bioreactor should be well sealed. Otherwise, for example, wild yeast will break in there and will consume our microorganisms' nutrition. Then after some time taking a look at what is growing in the bioreactor, we will see that our fine microorganisms are not there anymore, but the bioreactor is full of the wild yeast. Therefore - everything has to be hermetically sealed! If you want to take samples during the process, it should be done in a way that none of the "bad" microorganisms could get into the bioreactor vessel. Microorganisms will not reproduce everywhere you want. They acknowledge only stainless steel, good glass, teflon and, perhaps, some inert material. In the inner part of the reactor there cannot be ordinary zincified screws, also sensors with brass parts should not be used. Also very important is that before microorganisms start "living" in bioreactor, it has to be well cleaned and washed, and any survivals of „bad" microorganisms have to be annihilated. It can be done is by using annihilating sterilization.

Bioreactor's preparation for operation

In order to realize the cultivation of microorganisms it is necessary:

1. To prepare the bioreactor for cultivation;
2. To sterilize the bioreactor;
3. To prepare the inoculum.

Preparation of the bioreactor for cultivation means that the bioreactor vessel and the required hoses and tubes are cleaned and washed, all of the sensors are installed in the bioreactor, and other devices (pumps, titrated flasks, etc.) required for the process are connected. The bioreactor vessel, its hoses and tubes should be sterilized to avoid possible infectious (non-sterilized) disease breeders. Depending on the bioreactor's construction and volume one of the following sterilization methods is used in the laboratory bioreactor:

1. Autoclaving - installation of the bioreactor vessel, its hoses and tubes (which are disconnected) in the autoclave.

2. Sterilization in the place - steam supply to the bioreactor's jacket. In this case, the bioreactor is not moved somewhere else, and the bioreactor jacket is usually connected by a 3-way valve to the steam line pipes.
3. Sterilization in situ - the heating of the inner part of the bioreactor vessel is provided by the heaters present inside the bioreactor. In this case of sterilization, the glass part of the bioreactor vessel should be protected with a metal jacket.

Variant 1 is commonly applied for glass or steel/glass reactors up to 10 liters, as the installation of a larger reactor in the autoclave is difficult.

Variant 2 is commonly applied for steel and steel/glass reactors with a steel jacket.

Variant 3 is applied mainly for bench-top type reactors with the powerful enough built-in heaters, a thermostat or a steam generator. This variant is commonly applied in the corresponding reactors up to 20 liters.

During the sterilization, the bioreactor and the corresponding gear should be hermetically sealed to avoid the penetration of the microorganisms wandering around in the environment.

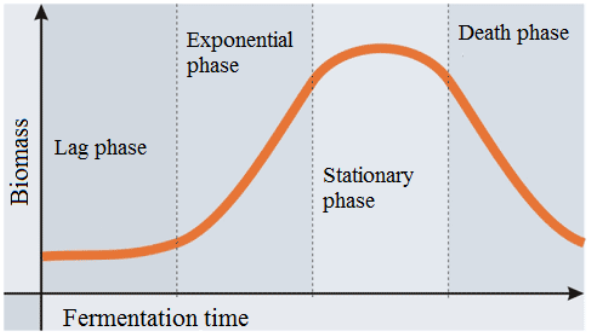
The nutrient medium can be sterilized separately and then under sterile conditions fed up (for example, with the help of a peristaltic pump) in the bioreactor. The nutrient also can be first fed up in the bioreactor and then be sterilized along with the bioreactor.

Growth of the Microorganisms

The microorganism cultivation process, fermentation, starts with the moment when the preliminary prepared inoculum (in a flask or other smaller bioreactor) is fed up in the bioreactor under sterile conditions.

The reproduction of the microorganism culture is characterized by 4 phases in time cycles:

1. Lag phase;
2. Exponential phase;
3. Stationary phase;
4. Death phase.



During the lag phase, the cell metabolism focuses on synthesizing enzymes in a definite reproduction nutrient. The lag phase length can vary also for the same microorganism culture in a definite nutrient medium, since it depends on different factors, e.g. on how many non-growing cells are present in the inoculum.

The exponential phase is the growing period in which the cell division with the logarithmic increase in the population number occurs. Such a dramatic increase in the growth rate is a limited period of time in a fixed amount of the nutrient. The nutrient resources are eventually exhausted or the process is inhibited by the separation of the toxic metabolite.

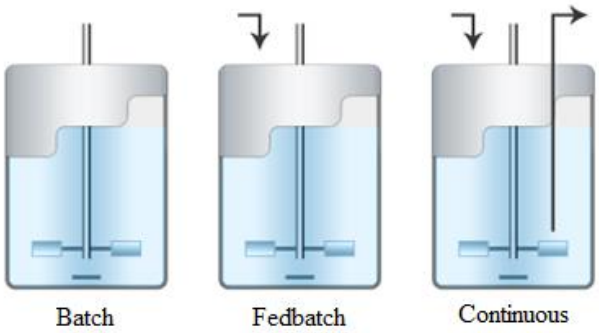
The growth stops, and the so-called stationary phase sets in, although the cell metabolism goes on acting, and the process of separating the secondary metabolite can begin. In many cases, the aim of fermentation is the obtaining of the secondary metabolite rather than biomass, since the former can be used as the raw material for obtaining valuable products and preparations. In this case, the fermentation is purposefully maintained at the stationary phase.

If the fermentation is continued for some time in the stationary phase, then a gradual decrease in the cell activity, i.e. the death phase can begin.

The batch fermentation process duration can vary from about 8 h till 5 days. It mainly depends on microorganisms strain, substrate art and fermentation task.

In terms of realization principle, there are 3 types of the cultivation process:

1. **Batch;**
2. **Fedbatch;**
3. **Continuous.**



In the **batch process**, the bioreactor is supplied with a fresh nutrient and thereby the inoculum is fed up there. At the end on the fermentation process, the content is passed to the separation stage, the reactor is cleaned and sterilized to be ready for the next process.
In the **fedbatch process**, a fresh nutrient (the feeding up intensity is commonly connected with the growth or biosynthesis rate) is supplied in the bioreactor continuously or in portions. When the bioreactor is full, it is partially or completely discharged. The process is finished or resumed.
In the **continuous process** or chemostates, the solution cultivated in the bioreactor is continuously discharged. The continuous process can proceed for a very long time, and its duration is commonly determined by the production requirements and technical factors.

Most widespread is the fermentation with fedbatch, and the latter is commonly applied for biological products. In this case, the drawbacks of the batch process are prevented with minor technical changes.

Continuous processes or chemostates most frequently are applied for large-scale production of biochemicals. From the viewpoint of production, such processes are more economic, although essential technical modifications as well as a deeper insight in the given fermentation kinetics are required.