Basic design of laboratory bioreactor

In terms of the construction, the following variants of the laboratory bioreactor can be made:

1. Glass bioreactor (without a jacket) with an upper stainless steel lid.
2. Glass bioreactor (with a jacket) with an upper stainless steel lid.
3. Glass bioreactor (without a jacket) with the upper and lower stainless steel lids.
4. Stainless steel bioreactor with peepholes.

Lid (upper)

The bioreactor's upper part has:

1. Ports for electrodes (pH, pO_2, T, p).
2. Ports for the supply of the titration and feeding medium.
3. Pipes for sampling and chemostate.
4. Ports for the connection of the outlet air condenser and filter.
5. Drive sealing (if the mixer's upper drive is used).
6. Air sparger mounting.

The lid's ports, connections and mountings should ensure the air-tightness and leak-proofness, sterility, convenient removal and installation of the sensors and other elements. The lid is usually connected with the reactor's rest part using special screws with a circumference diameter (4-8pieces) and a flat or "0" ring silicone rubber sealing.

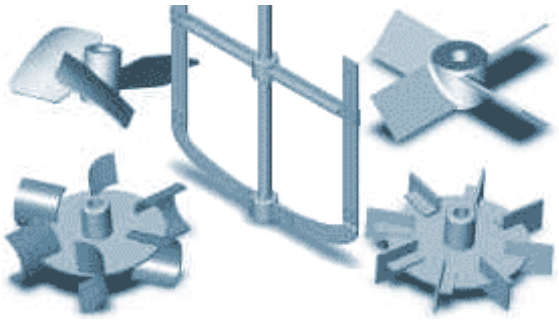
Lid (lower)

If the bioreactor has a lower lid, then the following ports and elements should be placed and fixed there:

1. Discharge valve;
2. Sampling device;
3. Sparger;
4. Mixer's lower drive;
5. Heaters.

All these connections should secure air-tightness and leak-proofness and should prevent the conditions for infection agents reproduction ("pockets", unevennesses, etc.).

Mixers



Practical principle of mixers application

The mixer is mechanically "put on" the mixer's axis. The mixer's diameter is normally 1/3 - 1/2 from the diameter of the reactor vessel. The location of the mixer's axis depends on the fact whether the bioreactor has the upper or lower drive. From the constructive viewpoint - the upper drive is realizable more easily.Also the construction of the sealing is easier than in the case of the lower drive. And servicing of the bioreactor is simple as well then: the motor is disconnected from the sealing connection places, and then the lid, together with the mixer axis and the mixers, is taken off. In its turn, the lower drive provides a whole range of attractions connected with the operation regimes. In this case, mixing can be ensured in the bioreactor at the removed upper lid. This is important when modelling mixing as well as when cleaning and washing the bioreactor. In upper drive case, it is also easier to optimize dispersion, as the mixer can be close to the bioreactor's bottom.

The standard Rushton turbine is most popular

The standard Rushton turbine is the most widespread mixer's construction. Its diameter *d* is 1/2 - 1/3 (most typically, 1/3) from the vessel's diameter. The Rushton turbine is a typical radial flow generating mixer. For the mixer axis there are commonly 2-3 standard turbine mixers. The given mixer ensures the highest input power at fixed rotational speed values of the mixer (power index KN = 5-7). This mixing system secures a sufficient course of mixing intensity, bubble dispersion and other mixing operations and provides the highest introduced power by constant rotations speed, comparing with other mixers. Therefore, the most of bioreactors is equipped this type of a mixing system.

The mixing of mechanical sensitive microorganisms

However, in the practice of microorganism cultivation, there are fermentations in which the application of the standard turbine mixer does not provide the optimal cultivation results, as irreversible mechanical damages of the microorganisms' cells are caused (this applies mainly to mycelial organisms). For mixing of mechanically

sensitive mycelial microorganisms, mixing systems are recommended, that generate dominating axial flows, thereby ensuring a more even mixing throughout the reactor's volume. *EkatoIntermig* mixing systems are among the most widespread ones. The so-called "Counter-flow mixing system" is known. The approach to the mixing of more sensitive cells (tissue culture, animal cell, etc.) has to be different, because in this case, the mixing regime should have a laminar character.

Mixing at the big airflows

Another case, when the standard turbine is not effective enough,is the dispersion of a great air flow at relatively low rotational speed values of the mixer. With increasing the inlet air amount, it is not dispersed any more, as the "flooding" regime sets in. In this case, to increase the dispersion efficiency, it is recommended to apply SCABA AB 6 SRGT, Chemineer Inc. CD6 or BT6 mixers. The efficiency of these mixers, in comparison with the standard turbine mixer, becomes even more pronounced in the cases of three-phase flows (intensive aeration and the presence of dispersive particles in the medium).

Baffles

Baffles are vertical radially located plates (their width is about 10% from the bioreactor vessel diameter). There are normally 3-4 such plates in the reactor. Baffles are necessary to prevent the formation of a funnel. As a result of the funnel formation, the maximal possible rotational speed of the mixer can be essentially limited. By using baffles, the consumed electric power of the mixer increases by about 20% at the same rotational speed.

Sparger

Compressed air is supplied through a sparger into cultivation medium. The most widespread constructive solution is a loop pipe with small holes in the lower part (d = 0.05 - 0.15 mm). For mycelial culture fermentations, also loop spargers with a conical air outlet channel are used. This construction prevents the possible overgrowing of spargercracks, because in this case, the outlet diameter is greater.

Bioreactor vessel

Depending on the construction type, the bioreactor vessel is made of steel, metal or their combination. The relation between the height H and diameter D of the bioreactor is within 1.5-2.5. The bioreactors produced in Europe are commonly more "stately" than those produced in the U.S.A. The reactor filling is about 70%. Thus, it is also the working volume in the reactor vessel. There are high requirements for the reactor vessel materials to prevent the inhibition of the microorganism growth. The same

applies also to any other part (sensors, pipes, etc.), which are installed inside the bioreactor vessel. The glass should be 100% borosilicate, e.g Pyrex® and Kimax®. All the metal parts should be made from stainless steel. The most widespread brand of the stainless steel applied in bioreactors is 316L. The letter “L” indicates that this steel is with a low composition of carbon. The inner surface of the stainless steel bioreactor should be polished to about a mirror surface quality to facilitate the washing and sterilization process. Welding should be carried out in a fully inert gas environment. Argon should be used as the inert gas. Inadequate welding technology application may eventually lead to the corrosion of the welds.

Condenser, outlet valve and filter

The condenser returns the vaporized water back into the bioreactor and prevents filter from clogging. For this purpose, there is a pipe that is wounded inside of the condenser (or another solution that ensures, as possible, a longer air pathway); outside, there are two ports for cold water supply. The condenser is usually connected to the port located in the cover's centre. The condenser outlet is connected with the outlet valve. With the outlet valve, it is possible to regulate the necessary overpressure in the bioreactor's vessel. The valve outlet can be connected with a porous microbiological filter.

Sampling

On the one hand, sampling may seem to be a simple procedure - just open the manual valve in the inlet of the bioreactor vessel, supply as much fermentation broth as required for the sample, and close the tap! In this way of sampling, we can guarantee that infection will not be avoided.

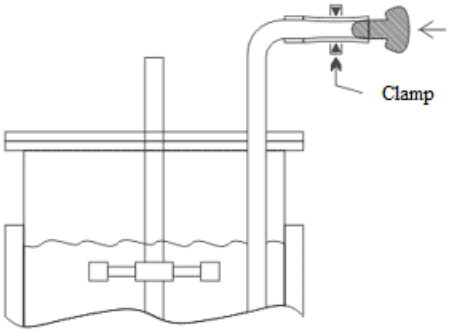
The sampling construction has to be designed in a way that prevents non-sterility before and after the sampling. In the sites of the infection origin, sterilisation should be performed promptly with alcohol or steam. There are sampling constructions of different companies that are offered for sale:

- www.keofitt.dk
- www.alnab.se
- www.strahmanvalves.com

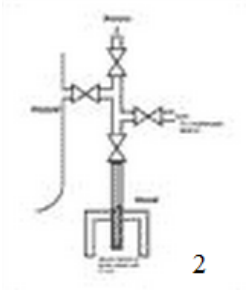
The essence of sampling is based on one of the following principles:

The first variant is simple sampling line. A bladder made of silicone or a similar material is placed on the sampling pipe, and its end is closed with a clamp (see the illustration on the right). Thereby, it is sterilized together with the bioreactor vessel, and it remains in such a state until the sampling. When sampling, the clamp is

removed, and the bladder is also pulled down. With the sample's discharge, the pipe's end is immediately washed with alcohol. Then, in a similar way, the sterilized bladder is put on (doing so, sterility has be observed certainly). This method is applicable if the given fermentation does not have very high demands on sterility. Another drawback of this method is a hampered possibility of choosing the sample's amount.



Variant 2 and 3 are showed in the following figures:



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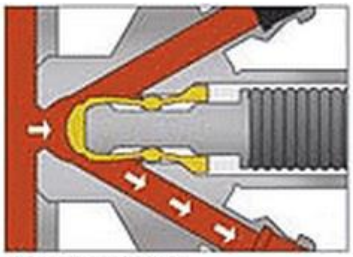

Fig. 1 Open Valve (sampling)

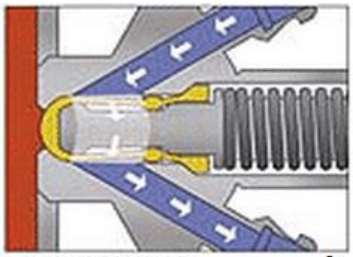

Fig. 2 Closed Valve (sterilizing)

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In principle, variant 2 can be realized by the laboratory staff. Variant 3 is a compact, and seems to be elegant and a simple solution, but for its realizing high accuracy and professionalism is required. A wide range of industrial sampling valves operates according to this principle.