Main operation requirements for bioreactors

The main operation requirements for bioreactors can be divided into 3 groups:

1. Sterility;
2. Mixing/aeration as an essential mass exchange factor;
3. Process monitoring and control.

To fulfill these requirements, the bioreactor design should be executed with the corresponding professionalism, and its technical performance (surface treatment, welds) should be of high quality.

Sterility

One of the major quality comparative characteristics of different bioreactors is the ability to provide sterile fermentations. These requirements are determined to a great extent by the bioreactor's construction and the surface treatment quality. All the further regarded non-sterility risk factors are mainly connected with securing of hermetic sealing at different stages of the process and surface treatment quality. The most characteristic possible non-sterility risk factors from the viewpoint of the construction are as follows:

1. Mixer's stuffing-boxes, sealing of the sensors and other devices' inserting ports

   One of the most typical pathways for infectious diseases entrance into a bioreactor is the mixer's stuffing-boxes. It is not always easy to provide the effective sealing and unhindered rotation of the drive. Apart from this, the regular servicing of the driver's stuffing-box should be ensured to avoid the accumulation of the infection there. To prevent problems connected with the stuffing-box, bioreactor drives are constructed on the principle of magnetic drives. In this case, the torque is transferred with the help of the magnetic field. As a result, the bioreactor vessel can be fully sealed. When sealing ports of sensors and other devices (supply of the titrated and feeded up components, sampling, chemostate implementation, etc.), it should be taken into account that sealing only with the force of the operator's hand has to be possible, and also you needs to be sure that the sealing properties would not change as a result of the sterilization temperature.

2. "Pockets", unevennesses and other weaknesses for the accumulation of infection inside the bioreactor vessel.

   Inside the bioreactor, infection can be accumulated in spots where irregularities and unevennesses take place. In such spots, infectious microorganisms can "hide themselves". Therefore, the bottom inside the reactor should be rounded off, there should not be acute angles, and the surfaces should be polished.

3. "Unconsidered" sampling, methods and construction

   When taking samples a steam, flame and other conditions that ensures sterility should be provided, so that after the termination of the sample spurt, the infection "would not manage" to get into the fermentation solution.

4. Filtration of the inlet and outlet air flows.

   Air should be supplied into the bioreactor through the appropriate porosity air filter so it could hold up the possible infection source. The inlet air flow before filtration also can be supplied through the pipes, which are being heated. Thereby, by the thermal action, an attempt is made to combat the possible infection at least partially.

5. Maintenance of overpressure.

   It is important to maintain the overpressure (0.2-0.5 bar) in the bioreactor's upper space (i.e. between the fermentation solution and the cover of the bioreactor) to ensure the protection against the income of infection. The infection income through the outlet air line is hampered by using the outlet air filter.

6. Even and effective heat transfer.

   It is necessary to provide the sterilization temperature with energy consumption even as possible and also to provide that heating would be even. If the heating inside of the bioreactor is not even, there is a risk that there will be zones inside of the vessel with insufficient sterilization temperature.

Besides this, during the sterilization process the sensors, devices, connections and other units should not lose their properties. It means that only sterilizable sensors (i.e. those which do not change their properties after the effect of sterilization) and rubber or other materials, with working temperature at least 150°C, should be used for sealing.

Mixing/aeriation as an essential mass transfer factor

Mixing and aeration are not the only factors that determine what mass exchange there will be in the bioreactor or how the microorganisms will grow. It is determined both by the properties of the microorganism strain and the choice of the balanced nutrient, process regime, etc. Let these factors remain for technologists, but we will discuss what will be profitable for the bioreactor in this respect.

Regarding to the evaluation of the role of mixing, the 2 extreme points of view are rather widespread:

- Microbiologists often say: "Why would mixing have an essential role in fermentation process? Everything is simple - if the partial pressure of the dissolved oxygen (pO_2) is too low, then more intensive mixing is required. And if the mixer's revolutions cause problems with coping with foam and other disorders, then we simply do not increase mixing and consider pO_2 as insufficient".

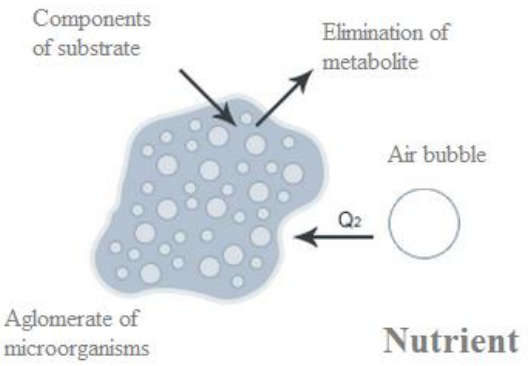
- While "mixing people" say: "Oh, it is not so simple with mixing! Firstly, if incorrect mixing and aeration regime is chosen - the mass exchange at the same input power is decreased dramatically. Secondly, the choice of the incorrect mixing system already at a minor input power ("mixing people", unlike microbiologists, usually do not say "mixer revolutions" to characterize mixing, but rather use "introduced power with mixer") can cause irreversible mechanical damages for sensitive microorganisms. Thirdly, the pointless increase of air consumption causes the worsening of mass exchange". And there are at least 6 more arguments that states how important is mixing in microorganisms' cultivation.

But what is the truth after all?

In order to clear that up, first of all, let us enumerate the results of mixing:

- Air bubble dispersion;
- Mass transfer from air bubbles (i.e. oxygen supply) to the liquid and then to cells;
- Supply of the nutrient components to cells (more precisely, cell agglomerates);
- Prevention of sedimentation;
- Providing the heat transfer;
- Solubility of the nutrient's components which are less soluble.

The mass transfer process during the cultivations of microorganisms is explained in the following picture:



As it has been already mentioned in the division "Basin design on laboratory bioreactor" the most widespread type of mixers are standard Rushton turbine. At a constant rotational speed of the mixer, they ensure the highest input power. This is very practical from the viewpoint of cultivation regime selection. Further it will be shown that there are, however, fermentations, in which the standard turbine is not the best solution. Certainly, there is a fermentation in which the role of mixing is relatively trivial. However in these cases as well different mixing/aeration regularities should be taken into account:

1. <u>Minimal and maximal limit of the mixer rotation.</u>

   Irrespective of the pO_2 values (or other alternative growth or respiration parameters), it is not recommended to choose a mixer rotation speed lower than the empirically determined critical limit n_{min}. This limit, n_{min}, is chosen so that the following would not appear:

   - Sedimentation;
   - "Died" zones.

   While, the choice of the critical limit n_{max} of the mixer's maximal rotational speed is determined by the following phenomena:

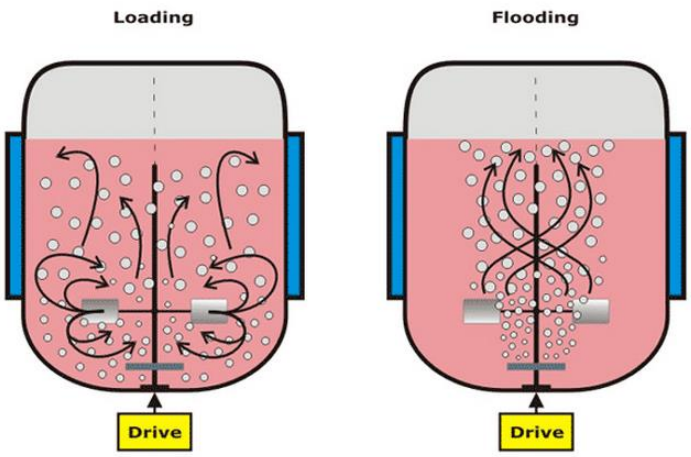
   - Foaming;
   - Liquid surface fluctuations, i.e. "waving", hence, also the liquid's evaporation.

2. Mixing/aeration relationships.

When choosing the mixing and aeration intensity values and their relative mutual interactions, the following should be taken into account:

- To increase the intensity of oxygen and other components' transfer intensity, first of all, we recommend to start with the mixer rotational speed increase, and, only when $n > n_{max}$ begin gradually increasing the air amount Q that is necessary for aeration. Before that, Q value is chosen to be enough to provide a stable aeration. Normally it is 1 vvm (vvm - amount of the introduced air versus the bioreactor's working volume). It means that, if we define air consumption in l/min, then the amount of the introduced air Q will be the same as the bioreactor's working volume.

- At relatively low rotational speed values of the mixer, the increase in the amount of the supplied air should be avoided as far as the "flooding" effect begins. What the "flooding" effect is and how the transfer in it from the "loading" state occurs will be explained by the following illustration:



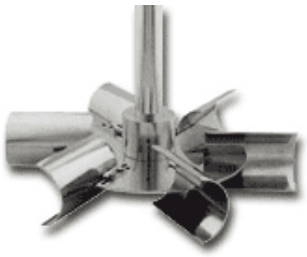
As it can be seen in illustration - as the "flooding" regime sets in, air bubbles are concentrated only in the middle part of the reactor, and they are poorly dispersed. Hence, such a mixing/aeration state is very undesirable for the microorganism growth.

It should be noted that the transfer from "loading" to "flooding" has a hysteresis nature. It means that, as the amount of the introduced air Q decreases, the "flooding" effect disappears at air consumption than the starting one.

It should be noted that, as the "flooding" effect begins, the standard Rushton turbine is not the most suitable mixer's variant any more. In these cases, the most appropriate mixing systems are Chemineer CD-6 or BT-6, as well as Scaba AB 6SRGT (also called the Smith mixer). Thereby, the mixer

construction ensures an intensive grabbing of air bubbles in the radial direction also at minor rotational speed values of the mixer. As a result, the air bubbles cannot do anything else but to obey the dispersion. Using this type of mixers, it is possible to essentially increase the air amount Q at which the "flooding" effect sets in.

3. Mechanical cultivation of sensitive microorganisms



In this section mainly the cultivation of mycelial microorganisms will be described, because mixing of more sensitive cultures has other aspects, i.e. the cultivation of these cultures even in the minimal turbulent regime is not permitted.

Mixing mycelial fungi microorganisms with a standard Rushton turbine, the mass exchange in the cultivation process increases only up to a definite rotational speed of the mixer, and, with the further increase in the mixer's rotational speed, the mass exchange parameters even begin to get worse. The reason for this phenomenon is the irreversible mechanical damage of the cells. Of course, this critical rotation of the mixer is not strictly fixed and depends on different factors:

- Microorganisms' strains;
- Nutrient's composition;
- Aeration regime;
- The amount of the grown biomass (at a greater biomass, the critical rotational speed of the mixer usually decreases, because in this case, it is more difficult for mycelial microorganisms to "run away" from the locally intensive mixing zones);
- And other factors that determine the medium's rheological properties and cell condition.

Ekato Intermig is one of the most widespread mixers for mycelial cultures. This type of mixers consists of two mixers - the lower and upper one. In this combination, axial flows are generated in the mixer, and the mixer's radial end construction ensures a sufficient radial mixing. Thereby, an even distribution of the energy introduced by the mixer throughout the bioreactor's volume is ensured, resulting in the decrease of the maximal shear forces.

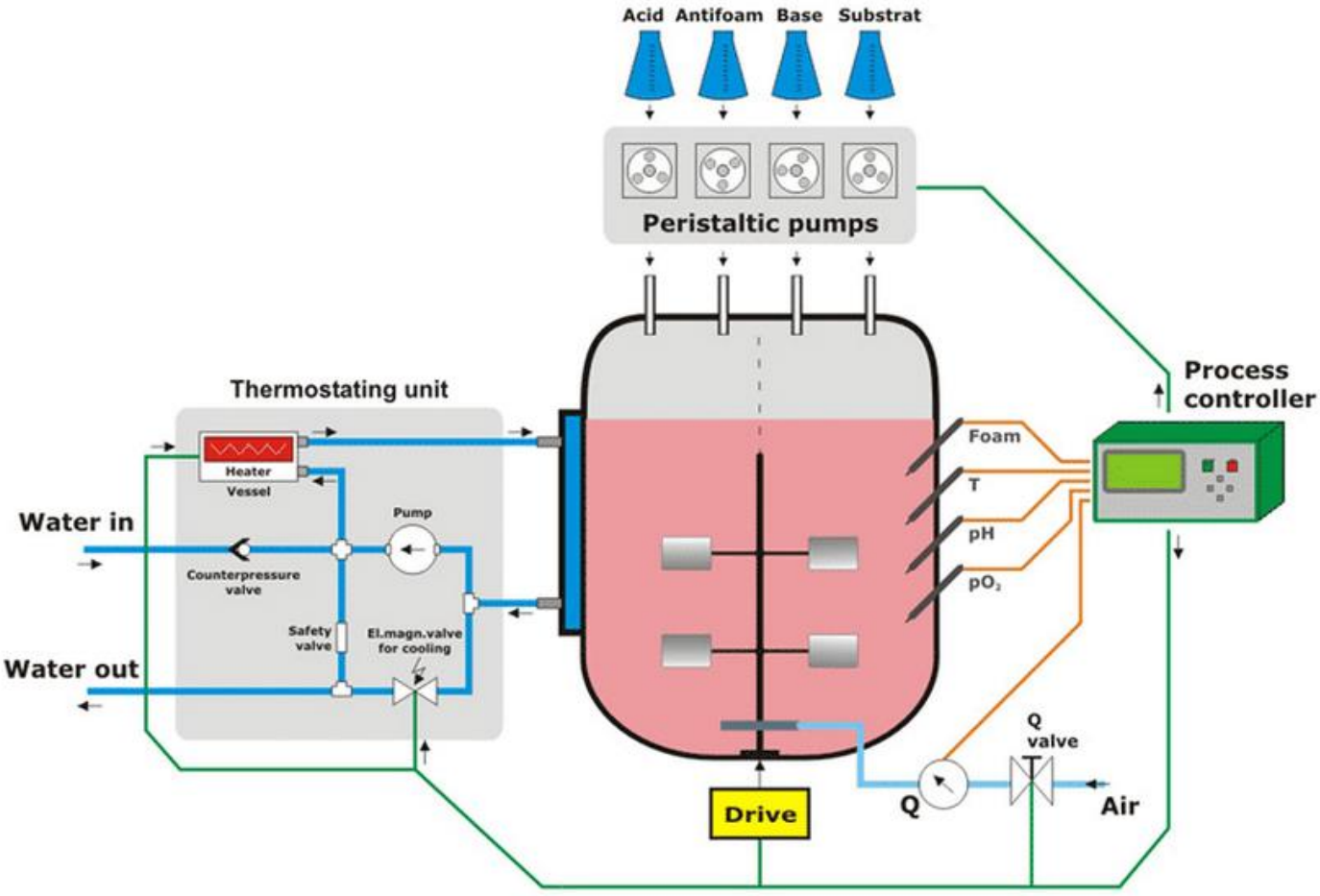
Another construction solution for mixing mycelial cultures is the so-called "counterflow mixing system". In this mixing system, the lower mixer generates an

axial flow upwards, while the upper mixer - downwards. The form of the mixer's blades ensures a sufficient flow eddy, i.e., as a result of tangential components, the given mixing system ensures both even distribution of the mechanically introduced energy (i.e. low shear stresses) and also a sufficient dispersion degree.

Process monitoring and control

The integral part of a high-quality bioreactor is a process controller. Such controller usually is specially designed for a definite bioreactor brand. This is rather connected with the fact that microorganism cultivation processes have relatively high requirements in respect to precision and sophistication. All this is despite the fact that almost all bioreactors monitor and regulate the same values actually invariably.

The monitoring and control scheme of a typical fermentation process is shown in the following illustration:



Temperature, pH, pO_2 and foam are the parameters that are usually controlled and monitored in bioreactors.

Temperature

Temperature is an important parameter of fermentation, since, in the cultivation of many microorganisms, the temperature deviation by a couple of degrees can diminish dramatically the growth and biosynthesis productivity. The cultivation temperature is commonly monitored with an accuracy at least ± 0.5°C. For temperature measurements, stainless steel Pt100 sensors are normally used. The temperature in laboratory bioreactors is controlled by one of the following ways:

1. A heater is located inside the bioreactor vessel, and cooling is ensured by thin-wall pipes located in the upper cover, which are connected with an electromagnetic valve with the cooling water.
2. Heating and cooling proceed in a thermostat, and this thermostatted water, with the help of a pump, circulates through the bioreactor jacket.

Variant 1 is less complicated, and it ensures a more economic constructive solution. This variant works very well for small bioreactors with the volume up to about 5 liters. Variant 2 ensures a more even distribution of heat throughout the bioreactor volume, which is essential in cultivation of microorganisms.

In the temperature regulation process, the main reason for the regulation inaccuracy is the incorrectly chosen PID parameters. This manifests itself as temperature oscillations.

To regulate the temperature precisely, the main obstacle is often the too high minimal portion of the cooling water. So the valves in the cooling water supply line should be adjusted correspondingly. Another factor for the regulation accuracy is the area and density of the heat transfer surface, because if the inertia is high then it is difficult to reach higher accuracy.

pH

The control of pH is based on the comparison of the adjusted "set point" and pH real values. For pH measurement, practically only sterilizable electrodes (most often "Mettler-Tolede" electrodes) are used. The control of pH values is ensured with the help of peristaltic pumps (silicone tubes are commonly used), correspondingly metering out the acid and the alkali. Normally, the "set point" adjustment consists of the lower pH_{min} and higher pH_{max} values. If pH is between these two values, then no influence occurs. Such an adjustment of the pH "set point" is applied to prevent the overdose of the titration solution. On the other hand, the "narrow" regulation limits of pH are not necessary for the successful course of the cultivation process. It should be mentioned that pH measurements should be accurate (± 0.02 pH units), since the dynamics of pH values' changes provides valuable information on the process kinetics.

pO_2 (partial pressure of dissolved oxygen)

One of the most specific aspects of the fermentation monitoring is pO_2 measurement and control. pO_2 control is characteristic only for fermentation processes. There are different pO_2 control principles:

1. Varying the mixer's rotational speed n, assuming that pO2 ~ n.
2. Combining the change of the mixer's rotation speed n and the amount of the inlet compressed air Q. It is assumed that pO_2 ~ n, pO_2 ~ Q. Usually n is regulated first until it reaches one of the limiting values - n_{min} or n_{max}, and its regulation is realized by varying Q.
   If n and Q have reached the limiting values, but pO_2 is not within the necessary limits, then the regulating effect does not occur.
3. Feeding up the substrate or one of its components. It is assumed that pO_2 is proportional to the feeding up intensity. Feeding up is normally realized with controlled peristaltic pumps. Sometimes this variant is combined with the regulation of the mixer's rotational speed n and the oxygen or air supply flow Q.

In pO_2 regulation, when adjusting the parameters, the following should be taken into account:

1. pO_2 is commonly adjusted in % from the fixed one. The adjusted pO_2 value has a lower and upper limit. The difference between both of these limits is usually 10 – 20 %.
2. Important parameters in pO_2 control are the control limits of the mixer's rotational speed n: n_{min} and n_{max}. It means that, when controlling pO_2, n will vary only within this range. These limits are determined in order to eliminate different undesirable phenomena:

    1. n_{min} choice is determined:
        a. to secure the minimal partly turbulent mixing level;
        b. by the guaranteed bubble dispersion;
        c. by the prevented sedimentation.

    2. n_{max} choice is determined by:
        a. setting in of the intensive foaming regime;
        b. irreversible mechanical damages of cells;
        c. liquid surface fluctuation and evaporation.

Foam

The appearance of foam is a very undesirable phenomenon, because during its appearance, there is a risk to lose an essential part of the fermentation broth. While

there is foaming, it is not possible to perform high-quality analyses and measurements. There are two commonly used methods or their combinations in order to eliminate foam:

1. Additional metering of antifoam, based on the information provided by the foam sensor. The given impulses are relatively low, with long pauses and a limited metering time. This additional control is necessary to avoid the possible overdose, otherwise the mass exchange parameters can decrease dramatically.
2. Mechanical metering of foam. For this purpose, an upper drive with a special disk-type or other type of the mechanical foam breaking mixer is installed in the bioreactor's upper cover. If an intensive foaming begins, then the mechanical breaking of foam will not help.

An optimal solution is the combination of both methods. The application of Variant 1 is more widely used in laboratory scale bioreactors.