BIOREACTORS – NEW SOLUTIONS FOR OLD PROBLEMS

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Abstract

A major effort has been invested in making bioreactors more efficient. The goal has been achieved in several steps. Firstly, a replacement of the traditional rotation movement by an oscillating up and down movement of stirring discs driven by an electromagnet yields several important advantages. A soft, whole volume movement of the medium results in optimal gas distribution throughout the medium and is also advantageous for cell growth. No vortex is formed and the elimination of baffles simplifies the construction of the bioreactor. A single silicone membrane efficiently replaces traditional seals and assures perfect sterility. In the case of extremely sensitive cells gas distribution tubing can be wound on a spiral fixed to the axis. The up and down movement facilitates the gas transfer and simultaneously provides a gentle movement of the medium.

Secondly, a new heat radiation system has also been introduced. A heating spiral in a gilded parabolic reflector has been placed under the bottom of the vessel. The IR rays efficiently heat up the culture without overheating it at any volume of medium. Several innovations allow the construction of high quality bioreactors at lower cost.

Introduction

Many different types of laboratory fermentors and bioreactors are used worldwide. The selection of a good quality bioreactor is not easy. Some advantageous parameters found in one product are cancelled out by other drawbacks in the same system. Good high quality bioreactors are very expensive. Even then ease of use is not assured. The question arises, whether it is possible to construct a bioreactor, which would satisfy most requirements. The most important are: high quality and easy to use, perfect sterility, precise measurement, control and recording of all important culture parameters and last but not least, the bioreactor should not be expensive!

Based on long personal practical experience in the field, we have tried to analyse the requirements and select technical solutions, which would lead to the satisfaction of the criteria presented above. In the following sections several important parameters will be briefly described and new solutions suggested.

Results and discussion

1. Sterility and Stirring

The most important quality of a bioreactor is its faculty to keep the inside of the reactor sterile from the beginning to the end of the cultivation including the harvesting. The sterility is affected by technical and even human factors. Experience shows that some bioreactors are easy to keep sterile while others are not.

Without any doubt the major problem is stirring, where the motor axis transfers its rotation to the impeller. The critical point is the passageway through the cover into the vessel. Different simple or multiple lip seals and mechanical seals are used to ensure this place is tight. Our experience with such solutions is that they are unsatisfactory, because they are sensitive to service and they sooner or later lead to contaminations.

The only satisfactory alternative is magnetic coupling. Magnetic coupling always assures good sterility. However, it also presents other problems such as the deposition of material between the rotating and stationary parts of the system. In cavities the medium and cells can accumulate, dry out and decompose. Furthermore, magnetic coupling is an expensive solution.

In small volume reactors – spinners, the magnetic field is used to move a magnetic rod to stir the medium. As the magnetic force decreases as a cubic function of distance, this system can be used only for small bioreactors with a low oxygen transfer rate (OTR). Moreover, if the stirring rod turns on the bottom of the vessel, cells are destroyed.

We could not find any satisfactory solution to this problem until it was realised, that stirring need not be rotative. If an oscillating up and down movement could be generated inside a bioreactor vessel, then a single elastic membrane could replace a mechanic seal very efficiently. The axis could be tightly fixed to

the membrane and the elasticity of the membrane would allow the up and down movement. In addition, an inexpensive electromagnet could be used instead of a motor. After some development work we have found the following vessel head to perform very well (Fig. 1).

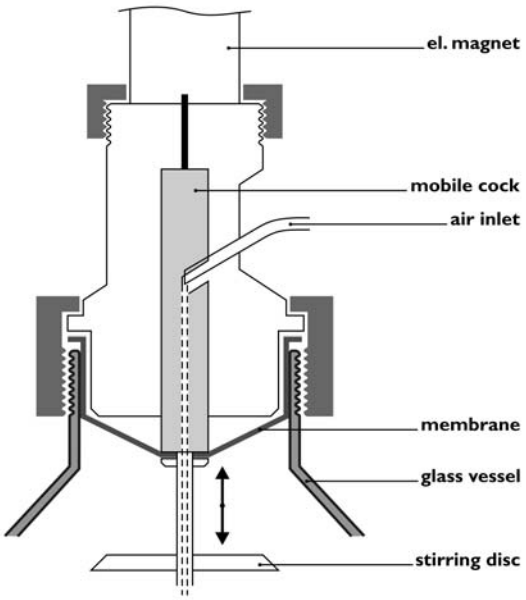

Figure 1. Fermentor head

The head is made of high performance, heat resistant epoxies. The stirrer axis and the central part of the membrane are tightly screwed to a mobile cock, which is connected to the axis of the electromagnet. The larger diameter of the membrane is screwed between the rim of vessel and the head by a large screw cap. The membrane is made of high quality silicone. The electromagnet can be easily removed before sterilization because its axis is screwed into the mobile cock.
The advantage of this construction is the perfect sterility of the connection. The head is easily fixed to the vessel by a large threaded cap and can be done with just one hand. This is much simpler and faster than the fixing of commonly used stainless steel head plates on existing bioreactors. An additional advantage of this solution is the longevity of the membrane and its low cost. If the stirrer axis is a tube, the air can be easily brought under the lowest stirring disc. This leaves one port on the vessel free. The gas inlet is preferably made directly through the moving cock.
One or more stirring discs (Fig. 2) are mounted on the stirrer axis.

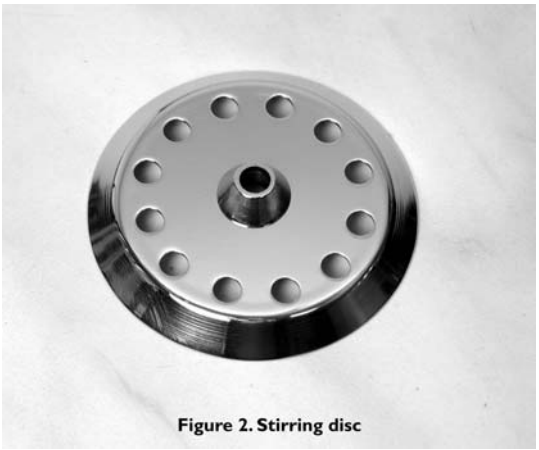

Figure 2. Stirring disc

A gas sparger is mounted on the lower end of the stirring axis. Air spargers frequently become blocked by salt deposition in some media. To eliminate this problem we designed a sparger in the form of a circular silicone membrane, which is perforated with miniature holes. They open more or less according to the gas flow rate. Possible deposits are expelled as they build up.

The bubbles are blown towards the bottom of the vessel without leaving any dead space (Fig. 3). Then, they are trapped under the second stirring disc. The fluid rotates in two rolls in opposite directions and distributes the air to the whole volume of the vessel as well as to the vessel walls. The third disc traps another portion of the gas and keeps it underneath for a short time. The vertical movement of the liquid and gas is obtained by perforated discs. The medium is well stirred without any dead zones. An additional advantage of this system of agitation is that no vortex is formed and therefore no baffles are necessary. Thus, the construction of our bioreactor is further simplified. The oxygen transfer rate is quite comparable to other stirred laboratory fermentors.

Figure 3. Stirring effect

2. Fermentor vessel

The concept of stirring by up and down vibration movement with a silicone separation membrane and a fermentor head calls for a fermentor vessel with one large threaded central neck and several side necks. Up to eight threaded side necks, similar to those used successfully in cell culture can be formed on the vessel. Most necks are of small diameter accepting common probes of 12 mm in diameter. One neck is larger and is used for sampling and addition ports. The necks are formed at such an angle that the medium is easily reached and the stirring discs are not touched. In this way, the external access to the probes and lines is not hindered and is much better than that experienced on the head plates of common bioreactors.
We decided not to use gaskets supplied with threaded caps, because the contact between the glass and a cap is limited. Instead we have developed large silicone stoppers (Fig. 4), which provide much better contact with the probes and to the glass walls. In addition, the silicone stopper is compressed when the cap is screwed on. This guarantees perfect sterility without the constant need of replacement, as is often the case for small O-rings.

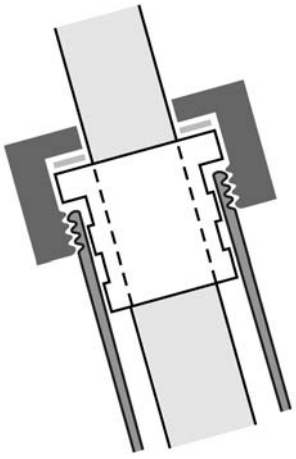

Figure 4. Stopper

In order to cover the most frequently used volumes in laboratory fermentors and bioreactors, we have selected vessels of 0.3, 1 and 3 l total volume (Fig. 5). The smallest working volume tested was 30 ml and the largest 2.5 l. It is possible to work with a recommended diameter to height ratio from 1:1 to 1:3. All vessels can be jacketed in their lower part.

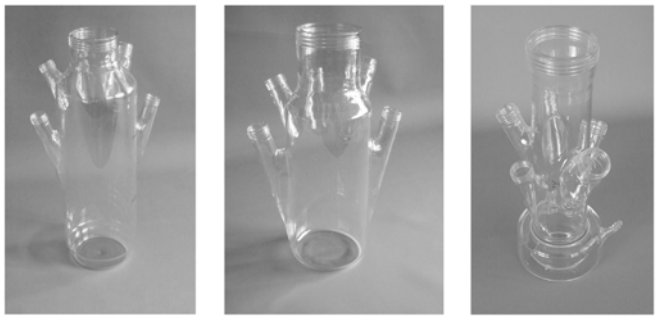

Figure 5. Vessels

3. Temperature control

Several different approaches are used in laboratory fermentors. The cheapest and simplest is a heating element placed directly into the medium. This option was immediately eliminated. There is a huge overheating on the surface of the heating element, especially with cultures of high optical density (OD). In our experience a temperature jump from 30 to 37 °C led to a partial carbonisation of the biomass as well as to a strong inhibition of growth due to the decomposition products formed.

A heating blanket as used on some fermentors today has been also eliminated, because it overheats the walls when the liquid level is lower than the upper edge of the blanket. Furthermore, it hinders the view into the reactor and requires a cable for electric current. We tried to eliminate any possible cable to make the surroundings of the fermentor more transparent and easier to monitor. Moreover, different heating blankets would be necessary for vessels of different size.

A circulating water bath has also been eliminated because of its cost, the necessity of using double wall vessels (this doubles the cost of the vessel) and the high thermal capacity of the bath. More time is required to stabilise the temperature after a temperature jump. On the other hand, the circulating bath poses no problems with overheating. However, it is necessary to clamp and disconnect the water lines before removing the vessel for emptying and cleaning. This is not practical. Circulating water baths are expensive, especially when they are refrigerated.

To solve these problems we have introduced a heat radiation system. The idea of radiation heating immediately appeared to be the solution of choice for laboratory bioreactors. If the source of radiation could be directed onto the vessel, then the heating would be as gentle as is the heating of water by the sun. The optimal location for the radiator is underneath the bottom of the fermentor vessel. Such a position leads to several advantages: There will be a natural convection even in a non-stirred reactor, no overheating of any part of the vessel, even with very low filling volumes, no added complexity to the vessel or to the space around it and finally electrical cables could be hidden in the base unit beneath the vessel.

It is known that Pyrex glass absorbs only about one half of the IR radiation spectrum. Therefore the second half of the heat will be absorbed directly through the medium. This makes the heat dissipation even more gentle. The gold surface was selected because it is the best reflector as it reflects more than 98 % of heat radiation. The reflector will not become hot and the heat transfer will be very efficient (Fig. 6).

The heating element of the radiator is made of Kanthal wire, placed in gilded parabolic reflector. Low voltage (5V DC) is required to eliminate any electrocution risk. Access to the hot spiral is impossible because during operation the glass vessel is placed above it. Since the spiral weighs only 10 g it has a very low heat capacity and temperature control is fast and precise. The heating effect is almost immediate and no losses of energy occur. Despite intensive heat radiating almost no light is produced to bother the operator. The radiator allows good heating and temperature control even in 3 l vessels. Medium temperatures over 60 °C can be attained. Another advantage of this bottom radiation heating system is that overheating is impossible, even at very low medium volumes.

Figure 6. Radiator

4. Casing

How to place the vessel in an optimal position? In principle there are two possibilities. The vessel can be integrated in the casing or free-standing. The advantage of a free-standing vessel is that a possible spill will not flow inside the casing and cleaning is easier. Access to all parts of the vessel is generally better than in incorporated systems. On the other hand, all tubing, supply cables and connections cannot be hidden in the casing and the complexity around the vessel is at its maximum. A special stand has to be constructed to keep the vessel stable on the table. A further disadvantage is that the free-standing solution requires more space. The electronics and other regulators are generally present as a pile of relatively large boxes beside the vessel.

In our opinion a system with an incorporated vessel has decisive advantages for small laboratory fermentors. The main advantage of the incorporated vessel is that the casing serves as a fixing structure for the vessel and the heating radiator can be conveniently placed underneath the vessel. The biggest drawback - presented by a possible spill getting inside the casing can be eliminated by modern sealing materials. These new materials, which are not completely polymerised and jelly-like, allow the fabrication of leak-proof seals that reseal perfectly after use.

Figure 7. Fermentor

We have selected a cascaded casing (Fig. 7). The lowest frontal part contains most of the electronics, the display and all the fermentor controls. It is tilted to improve the visibility of the readings. The vessel is fixed into an elastic ring on the first platform behind the front part and placed just above the radiator. The

elastic ring centres the vessel, attenuates the vibrations and reduces the noise. Two side holders adapt easily to the size of the vessel and assure a good hold on the vessel. The inclined segment in front of the last platform is used for stirring and probe connections and represents the shortest possible connection to the vessel. The last platform is used for the air output and the storage of bottles. To be able to place these bottles anywhere on this platform, special magnetic holders have been developed. Thus, up to seven bottles can be placed there simply and safely.
Pumps can be positioned on freely adjustable support plates fixed to two rods. In this way, we obtain good visibility of the tubing and connections as well as short connections. The elevated positions of the pumps eliminate a possible flow of the storage solution into the pump after a tubing rupture. This could happen if the stored solution is situated above the pump. Additionally, elevated pumps are easily visible and their status as well as their operation can be easily controlled. Finally, all other sockets are located on the rear side of the fermentor casing.

5. Control system

Most existing systems are modular. A very compact control system, which will allow good measurement and control of all important parameters by the same unit, is preferable for the user. Living organisms are extremely complex and it is desirable to measure and control as many parameters as possible. This will help to identify problems when they possibly arise. Therefore, we decided to measure and control the five most important parameters: temperature, pH, pO_2, airflow rate and stirring.
In addition, we wanted to have the option of controlling one supplementary parameter such as glucose concentration, optical density (OD) or the like. For this reason, one selectable and controllable parameter X has been included. All readings can be seen at a glance without the necessity of scrolling or selecting menus.

PUMPS

Inoculation, addition of acid or base and sample removal is carried out through four stainless steel capillaries equipped with Luer-Lock adapters (Fig. 8). It is advisable to use good pumps, which allow the use of a whole range of speeds going from 100 % to zero, together with the fermentor. When the difference between the preset pH and the real pH is large, the pump adds the acid or base at maximum speed. As the difference decreases the speed of addition decreases progressively. Hence, the desired pH-value is obtained rapidly and smoothly and is definitely better than switching on and off a pump with a constant speed.

Figure 8. Inoculation/sampling ports

INTEGRATOR

It is important to control the pH during cultivation. If the pH is recorded, only a monotonous straight line can be seen. It is more interesting to see how much acid or base has been added to keep the pH constant. Some producers supply electronic balances for this purpose. However, balances are expensive and take a lot of space on the bench. We have therefore constructed an integrator. The integrator allows a simple but precise integration of the amount of liquid transported by the pump. The electric impulses moving the stepping motor are registered and transformed into a direct current. The voltage can then be measured and recorded. This data yields important information about the culture growth, its kinetics and time of completion. The integrator can also be used for measuring enzyme activity (e.g. esterases, amidases, lactamases and other enzymes). It can be placed under the pump and does not require additional bench space.

6. Cell culture adaptor

The stirring by perforated discs provides a more gentle alternative in comparison with other stirring methods. At low frequency many types of cells can be cultivated with success. For very sensitive cell lines we have developed an aerating spiral. The gas passes into the medium through a thin wall of silicone tubing (Fig. 9). Because of the gentle up and down movement of the tubing wound on the spiral, the layers of oxygenated medium are rapidly displaced from the tubing surface. At the same time the medium is gently stirred over the whole volume.

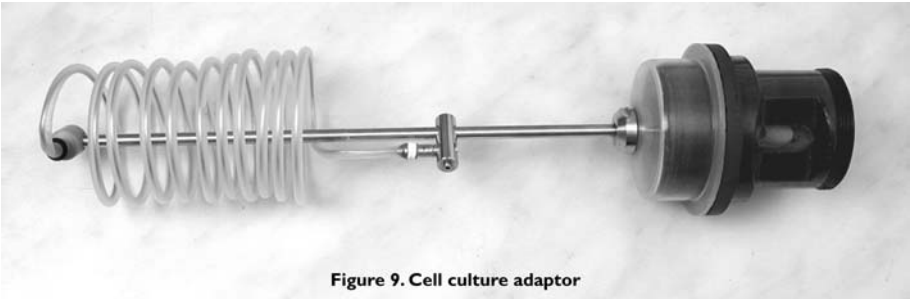

Figure 9. Cell culture adaptor

7. pH control in cell cultures

The pH of cell culture media is maintained constant by the controlled addition of gaseous carbon dioxide (CO_2). If the pH is too acidic, other gases, generally nitrogen, are blown inside to remove the excess of carbon dioxide.

For this purpose, we have developed a new controller system based on the mass flow measurement of the gas flow. The flow rate is regulated by a specially developed proportional needle valve, which is controlled by a microprocessor.

The connector of the CO_2 regulator is simply plugged into the acid pump socket on the rear side of the bioreactor. The system will take control of the gas flow as required for keeping the preset pH. If necessary, the nitrogen regulator can be plugged into the base pump socket of the bioreactor. This simple step ensures the regulation of the pH. This controller system replaces expensive gas mixing stations and provides much better parameters. The gas mass flow controller can be operated manually from the front panel or using a remote control. Flow rates and time intervals can be also programmed in advance. A fully digital control uses RS485 connection. Volume impulsions are generated after every 5 ml of gas flow. These impulsions can be totalised by the integrator, measured as a voltage and displayed on a recorder or on a PC (fermentation program FNet). In this way, exact volume information can be obtained.

8. PC control

Together with the development of the bioreactor we were keen to identify appropriate software, which would suit our new system. Again, we wanted a reliable and user-friendly program. This has been achieved by the modification of the existing industrial fermentation software SIAM. The Swiss firm SYSMATEC has developed new FNet software, which meets all our requirements. The software runs on Windows NT, 2000 and XP. Up to 6 fermentors, 12 integrators and 6 pumps can be connected to one PC. A detailed description of this software can be found on:
www.rhone.ch/sysmatec or www.rhone.ch/lambda.

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Figures