A test facility for fritted spargers of production-scale-bioreactors

C. Sieblist · M. Aehle · M. Pohlscheidt ·
M. Jenzsch · A. Lübbert

Received: 31 May 2010 / Accepted: 26 November 2010 / Published online: 12 December 2010
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Abstract The production of therapeutic proteins requires qualification of equipment components and appropriate validation procedures for all operations. Since protein productions are typically performed in bioreactors using aerobic cultivation processes air sparging is an essential factor. As recorded in literature, besides ring spargers and open pipe, sinter frits are often used as sparging elements in large scale bioreactors. Due to the manufacturing process these frits have a high lot-to-lot product variability. Experience shows this is a practical problem for use in production processes of therapeutic proteins, hence frits must be tested before they can be employed. The circumstance of checking quality and performance of frits as sparging elements was investigated and various possibilities have been compared. Criteria have been developed in order to evaluate the sparging performance under conditions comparable to those in production bioreactors. The oxygen mass transfer coefficient (k_La) was chosen as the evaluation criterion. It is well known as an essential performance

measure for fermenters in the monoclonal antibody production. Therefore a test rig was constructed able to automatically test frit-spargers with respect to their k_La -values at various gas throughputs. Performance differences in the percent range could be detected.

Keywords Fritted spargers · Bubble aeration · Oxygen supply · Animal cell bioreactors

Introduction

Most production reactors for recombinant therapeutic glycoproteins are aerated stirred tanks where hosts such as Chinese Hamster Ovary Cells are grown in aerobic culture (Wlaschin and Hu 2006).

In recent years nearly all of these cultures were aerated with air or an air/oxygen/carbon dioxide mix, dispersed into the culture by means of a sparger (Nienow 2006). Some reactor manufacturers prefer normal ring spargers with drilled holes, others simply use open tubes. Often, frit spargers (Gray et al. 1996), are used. All components in and around production bioreactors must be validated before they can be used in production, so the frit spargers. Here we are reporting about a test facility for frit sparger elements which are used in production scale bioreactors. We focus on the aeration characteristics of frit spargers. As the cell densities in production scale mammalian cell cultures are rather small (up to about 1.0×10^7

C. Sieblist · M. Aehle · A. Lübbert (✉)
Institute of Biochemistry and Biotechnology, Center for
Bioprocess Engineering, Martin-Luther-University
Halle-Wittenberg, Kurt-Mothes-Str. 3 / 320.2,
06120 Halle, Saale, Germany
e-mail: Andreas.Luebbert@biochemtech.uni-halle.de

C. Sieblist · M. Pohlscheidt · M. Jenzsch
Roche Diagnostics GmbH, Nonnenwald 2,
82377 Penzberg, Germany

[cells/mL]) and the concentrations of medium components of higher molecular weight are quite small as well, the media depict viscosities which are essentially equal to water (Nienow 2006). Hence, the gas dispersion tests are performed in a vessel filled with purified water conditioned sodium chloride on 300 mOsmol.

Frit sparging elements

Frits can be composed of Teflon®, stainless steel, glass and other materials. Most often they are formed from small solid particles (often spheres, seldom fibres) that are pressed into a shape and then sintered to form a stable porous wall. Usually, the sintered frits are mostly closed tube elements or as flat membranes. Figure 1 presents some examples of frit elements that can be fixed on a tubing system at the bottom of a cell culture bioreactor.

In the examples discussed in this paper the most commonly applied metal frit elements were employed. Frits are commercially available from many suppliers (Bohlender 2010; GKN 2010; ROBU 2010). Originally, these frit elements were constructed as filters, for instance as oil filters in automobile motors.

The main characteristic of the frit material is its mean pore size. The pore size depends on the size of the solid particles used to build the frit. As the solid material in the forms cannot be arranged regularly, the pore size distribution is rather broad. Also the necessary sintering process has a large influence on the porosity of the frits.

There are different norms classifying the frits produced for technical applications. Table 1 shows the pore size range classification by the most important norms, the ASTM and the ISO norm.

In biotechnology, one often uses coarse frits, which, according to the ISO norm depict pore sizes in the range 40–100 µm. This size distribution is a main reason for the fact that with such wide specification ranges we must expect very different gas dispersion characteristics even with frits from the same production lot.

When these frits are taken as gas spargers in bioreactors, we are mostly interested in their gas dispersion properties upon gas flows from the inner space through the frit-material into the liquid surrounding the frit element. Not all paths in the random particle arrangement of the frit material lead to a gas throughput, some are dead-ended, i.e., blocked; some are wider and some smaller (Fig. 2).

In animal cell culture reactors, the aeration rates are usually rather small. Cell lysis and foaming issues limit the sparging rates to about 0.1 vvm [volumetric flow rate per minute/volume of broth per minute] (Ozturk 1996). Hence, the test facility was designed for aeration rates according to this order of magnitude.

Qualitative inspection of the frits

As already mentioned, all frit tests reported were performed in water which is, with respect to the viscosities similar to the culture media (Nienow

Fig. 1 Frit sparging elements used in cell culture bioreactors. *Left* Teflon frit; *mid* stainless steel frit; *right* Al₂O₃ frit

Fig. 2 Pathways through a sintered material. Some pores are larger and some are smaller, some ways are longer and some are shorter

2006). However, in order to get reproducible results it is necessary to add salt to the water. The salinity could be chosen in such a way that the osmolality of the solution is in the range that is usually found in animal cell culture media. All tests reported here were performed in distilled water which was adjusted to 300 mOsmol by adding sodium chloride.

The gas dispersion properties, however, predominantly depend on the surface active media components. Currently, these cannot be adjusted in a sufficiently accurate way so that the conditions in given animal cell cultures can be met. Hence, it was decided to dispense with using surfactants and to perform relative comparisons between frit elements only.

Figure 3 gives an impression of the variability of the aeration behavior among the frits from the same production lot. Here, three frits were supplied from the same gas reservoir with the same air pressure. The entire gas throughput was 8 [L/min]. It is obvious that the gas sparging properties appear to be quite different.

At small gas pressures the difference in the bubble formation behavior is easier to see; however, the differences remain at higher pressure supplies. The general observation is that the gas bubbles do not appear homogeneously distributed across the frit surface. Also the bubbles disposing from the frit can be seen to coalesce in different ways.

Such a visual inspection of the bubble plume is quite informative; however, visual inspections cannot simply be made in a quantitative way. Validation of the frit performance needs a quantitative measure.

Fig. 3 Three frits from the same production lot, although aerated in exactly the same way, the three frits produce a much different bubble swarm

Quantitative performance measure

In order to find a representative measure, several possibilities were checked. First the pressure loss across the frits under controlled constant gas flow rates was considered. At the throughputs needed in animal cell cultures, the pressure loss was found to be about 5 mbar. If flow rate differences between different frits in the order of 5% are to be resolved, pressure measurements with an absolute accuracy of 250 μ bar at a pressure level of 1 bar would be required. This necessitates too a sophisticated measurement technique.

Measurement of the bubble size distribution could be made by video analysis techniques. However, such a technique would require an extremely expensive image processing development. Besides those, experiments would be limited for test on transparent containers and liquids.

Finally, k_La -measurements were performed. The mass transfer coefficient most directly characterizes the final aim of using a sparger; hence it is straightforward to develop a test rig based on dynamic k_La measurements.

For a dynamic k_La measurement we need an online measurement of the dissolved oxygen concentration

pO₂. In a first step the oxygen dissolved in the continuous liquid of the reactor must be stripped out with a gas not containing oxygen. Nitrogen is an obvious choice. In a second step, after all dissolved oxygen was stripped off; the gas supply must be switched over to air. Right upon the switch to air, the concentration of the oxygen dissolved in the liquid rises.

This rise in the pO₂ signal can be modeled and a fit to the measurement data gives the k_La value as the most important model parameter. As can be seen from the fitted curve segment in Fig. 4, after some dead time resulting from the change of the gas, the model fits quite well into the data.

Apart from small offsets with respect to time and signal amplitude, the data can be approximated by:

$$pO_2(t) = pO_{2(max)} \cdot (1 - e^{-k_La \cdot t})$$

In this way, k_La can be determined to a high accuracy with errors less than 1%.

The gas flow rates to the individual frits were controlled with thermal mass flow controller and varied around the values used in real cultivations. The dissolved oxygen concentration was measured with a fluorescence probe (Fibox3 AOT/Oxygen Sensor Spot SP-PS3-NAU-D5-YOP) manufactured and delivered by PreSens (Regensburg, Germany). As it depicts a small time constant $t_{90} < 4$ [s], it was preferred as compared with standard Clark-electrodes (e.g. Mettler-Toledo InPro6800), which showed a response time of $t_{90} = 90$ [s].

Figure 5 shows the k_La values obtained for different gas throughputs. From the scatter of the experimental data, the symbols, one can see that these measurements practically reveal a linear relationship between k_La and aeration rate Q_g within the aeration rate interval considered (linear regression coefficient 0.9948).

The usually applied correlation is

$$k_La_{\text{nitrogen}} = \sqrt{\frac{D_{\text{nitrogen}}}{D_{\text{air}}}} \cdot k_La_{\text{air}}$$

With the diffusion constants for nitrogen [$D_{\text{nitrogen}} = 1.88 \cdot 10^{-5} \text{ cm}^2/\text{s}$] and air [$D_{\text{air}} = 2.0 \cdot 10^{-5} \text{ cm}^2/\text{s}$] (from Cussler 2008) we expect a ratio of the corresponding k_La values of 0.9695.

The resolution of the measurements is so good that the k_La values of the oxygen transfer and the nitrogen

Fig. 4 Rising dissolved oxygen concentration upon aeration of the water in the test rig. Initially, a negligible dissolved oxygen concentration was in the water

transfers during the oxygen stripping procedure can be distinguished. Clearly, the difference is quite low, most often lower than the difference between different frits. Hence, the nitrogen curves can be used as redundant measurements of the oxygen mass transfer coefficients.

A most important requirement for test measurements is the reliability of such measurements. This can be tested by repeatedly measuring the k_La for the same frit in the same range of aeration rates.

Figure 6 depicts the results from 67 measurements repeatedly performed with one and the same frit. The data are close together and the most important distortion is that the test liquid becomes impure. This changes its rheological behavior. Hence, all five experiments, new water was taken. The message is thus: The k_La measurements at one and the same frit are highly reproducible. Hence, the first requirement to a validation procedure is fulfilled.

Fig. 5 Determined k_La values for air and nitrogen determined for single frit as function of the aeration rate

The next question is whether or not the technique is sensitive enough to distinguish between the different frits from one production lot.

Figure 7 shows the results from four frits arbitrarily chosen from one shipment.

The different frits can clearly be identified. Each leads to a separate line in the k_{La} -aeration-rate-plot. This does not only apply for oxygen. For nitrogen we obtain practically the same result. The clear message thus is: We can truly distinguish the different frits from one production lot by the k_{La} values they produce in the test rig. As the frits are employed in order to make gas–liquid mass transfer, we thus characterize the individual frits by their most important feature.

As expected, the influence of changes in the rheology is quite large. When Pluronic® F68 is added to the test liquid, the k_{La} is considerably dropping (Fig. 8). The measurements are still stable at these lower k_{La} values.

In order to make use of this technique in a commercial fermentation plant, the frit tests must not cost too much manpower. Hence, a test rig was constructed that allows to

- (i) easily insert a new set of frits and
- (ii) automatically start and perform the tests

In order to avoid problems with the test medium, all the frits are tested in the same environment.

Eight frits can be fixed at the bottom of the test column. These are connected to a gas pressure line via a buffer volume. Between the buffer and the frits, eight identical valves allow switching on or off the gas supply to any combination of frits.

Fig. 6 Repeated (67) k_{La} measurements performed at different aeration rates using one and the same frit

Fig. 7 Measurements of the k_{La} produced with 4 different frits from the same production lot as a function of the aeration rate

The entire arrangement is shown on the left side of Fig. 9. The gas supply as well as the electronics controlling the test rig is hidden in the box below the test column. On the right of Fig. 9, a schematic cut through the test vessel is shown. It depicts the frits screwed into bottom of the cylindrical test vessel together with the gas supply to the frits. All the frit elements are arranged equidistantly on a circle to provide the same conditions for all of them. By means of valves the individual frits can be switched on and off separately. In this way one after the other of the inserted frits can be used in the dynamic k_{La} measurements.

Finally it should be mentioned that the test rig can easily be used to characterize the sparger elements with respect to additional properties. For instance, the stripping rates for O_2 and CO_2 can be measured in this way. For all these experiments, the pH-value was kept constant.

All experimental results presented here were achieved in this piece of equipment.

Fig. 8 Influence of commonly used surface active additives such as Pluronic® F68 on the k_{La} measurement results

Fig. 9 Test rig for 8 frits to be investigated against each other. Each frit can separate be supplied with gas

Conclusion

In conclusion, automated k_La -measurements can be employed to test frits for their mass transfer performance. It was shown that the k_La value measured can be used as a quantitative performance measure. As shown in Figs. 3 and 7 such performance measurements are necessary as the delivered frits even from the same production lot differ significantly. The measurements are highly reproducible and the technique allows to clearly distinguishing between the different frits from a given consignment. A test rig construction was presented which allows convenient

tests of several frits simultaneously. Automation of the measurements was shown to reduce the workload of the tests significantly. The technique can easily be extended to the mass transfer of different gases that might be important in different cultivation systems.

Acknowledgments The help of K. Ostmann during the measurements is gratefully acknowledged.

Appendix

See Table 1.

Table 1 Classifications of porous materials

Nominal– Max. Pore size (ASTM)	Nominal–Max. Pore size (ISO 4793)	Fields of application
Extra-coarse 170–220 μm	P250 160–250 μm P160 100–160 μm	Filtration of very coarse materials. Gas dispersion, extractor beds. Support of other filter materials
Coarse 40–60 μm	P100 40–100 μm	Filtration of coarse materials. Gas dispersion, gas washing, gas absorption. Mercury filtration. Extraction apparatus
Medium 10–16 μm	P40 16–40 μm	Filtration of crystalline precipitates. Extraction apparatus. Removal of “floaters” from distilled water
Fine 4–5.5 μm	P16 10–16 μm	Filtration of fine precipitates. Mercury valve. Extraction apparatus

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