Acad. Sci. USA 83, 6255–6257
7 Martinek, K. and Semenov, A. N. (1981) *J. Appl. Biochem.* 3, 93–126
8 Martinek, K., Semenov, A. N. and Berezin, I. V. (1981) *Biochim. Biophys. Acta* 658, 76–89
9 Martinek, K. and Semenov, A. N. (1981) *Biochim. Biophys. Acta* 658, 90–101
10 Cambou, B. and Klibanov, A. M. (1984) *J. Am. Chem. Soc.* 106, 2687–2692
11 Zaks, A. and Klibanov, A. M. (1984) *Science* 224, 1249–1251
12 Deetz, J. S. and Rozzell, J. D. (1986) *The World Biotech Report* 1986 (Vol. 2) part 3, pp. 25–33, Online
13 Kirchner, G., Scollar, M. P. and Klibanov, A. M. (1985) *J. Am. Chem. Soc.* 107, 7072–7076
14 Fukui, S. and Tanaka, A. (1984) *Adv. Biochem. Eng. Biotechnol.* 29, 1–33
15 Fukui, S. and Tanaka, A. (1982) *Enzyme Eng.* 6, 191–200
16 Koshiro, S., Sonomoto, K., Tanaka, A. and Fukui, S. (1985) *J. Biotechnol.* 2, 47–57
17 Martinek, K., Levashov, A. V., Klyachko, N. L., Pantin, V. I. and Berezin, I. V. (1981) *Biochim. Biophys. Acta* 657, 277–294
18 Martinek, K., Levashov, A. V., Khmelnitsky, Y. L., Klyachko, N. L. and Berezin, I. V. (1982) *Science* 218, 889–891
19 Grandi, C., Smith, R. E. and Luisi, P. L. (1981) *J. Biol. Chem.* 256, 837–843
20 Larsson, K. M., Adlercreutz, P. and Mattiasson, B. (1987) *Eur. J. Biochem.* 864, 1–5
21 Klyachko, N. L., Levashov, A. V., Pshezhetsky, A. V., Bogdanova, N. G., Berezin, I. V. and Martinek, K. (1986) *Eur. J. Biochem.* 161, 149–154
22 Yoshimoto, T., Takahashi, K., Nishimura, H., Ajima, A., Tamaura, Y. and Inada, Y. (1984) *Biotechnol. Lett.* 6, 337–340
23 Takahashi, K., Tamaura, Y., Kodera, Y., Mihama, T., Saito, Y. and Inada, Y. (1987) *Biochem. Biophys. Res. Commun.* 142, 291–296
24 Matsushima, A., Okada, M. and Inada, Y. (1984) *FEBS Lett.* 178, 275–277
25 Takahashi, K., Ajima, A., Yoshimoto, T. and Inada, Y. (1984) *Biochem. Biophys. Res. Commun.* 125, 761–766
26 Takahashi, K., Nishimura, H., Yoshimoto, T., Saito, Y. and Inada, Y. (1984) *Biochem. Biophys. Res. Commun.* 121, 261–265
27 Takahashi, K., Nishimura, H., Toshimoto, T., Okada, M., Ajima, A., Matsushima, A., Tamaura, Y., Saito, Y. and Inada, Y. (1984) *Biotechnol. Lett.* 6, 765–770
28 Kazandjian, R. Z., Dordick, J. S. and Klibanov, A. M. (1986) *Biotechnol. Bioeng.* 28, 417–421
29 Grunwald, J., Virz, B., Scollar, M. P. and Klibanov, A. M. (1986) *J. Am. Chem. Soc.* 108, 6732–6734
30 Halling, P. J. and Macrae, A. R. (1982) European Patent Application 0064855
31 Macrae, A. R. (1983) *J. Am. Oil. Chem. Soc.* 60, 291–294
32 Klibanov, A. M. and Kirchner, G. (1986) US Patent 4601987
33 Nilsson, K. G. I. (1987) in *Biocatalysis in Organic Media* (Laane, C., Tramper, J. and Lilly, M. D., eds), pp. 369–374, Elsevier
34 Hammond, D. A., Karel, M., Klibanov, A. M. and Krukonis, V. J. (1985) *Appl. Biochem. Biotechnol.* 11, 393–400
35 Barzana, E., Klibanov, A. M. and Karel, M. (1987) *Appl. Biochem. Biotechnol.* 15, 25–34
36 Randolph, T. W., Blanch, H. W., Pravsnitz, J. M. and Wilke, C. R. (1985) *Biotechnol. Lett.* 7, 325–328

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Gas hold-up measurements in bioreactors

Joep J. M. Hofmeester

The gas hold-up of a gas-liquid dispersion is an important parameter in the fermentation industry. If it is too low, or too high, productivity can be adversely affected. Gas hold-up in fermentors cannot be calculated from physico-chemical correlations and, therefore, must be measured accurately for each fermentation.

This article surveys a number of methods for measuring the gas hold-up in gas-liquid dispersions, making particular note whether these methods can be applied aseptically.

Gas hold-up (also called the void fraction) is the relative volume of gas that is present in the gas-liquid dispersion in a fermentor (Fig. 1). Gas in foam on top of a fermentation broth does not contribute to the gas hold-up. Only the submerged air bubbles are considered in gas hold-up. Gas hold-up determines the transfer of oxygen from the gas to the liquid phase. If it is too low, the production rate in most aerobic fermentations will fall. On the other hand, high gas hold-up should also be avoided. The gas fraction determines the maximum broth volume in a specific fermentor since it excludes productive liquid. The gas hold-up is, therefore, not only an important process design parameter but, since the total volume of a fermentor cannot be changed easily, it also has a large influence on productivity.

Although many correlations are available for gas hold-up in pure liquid, there is hardly any information on gas hold-up in fermentation broths. The main reason is that the gas hold-up depends on the properties of the broth (e.g., ionic strength, biomass concentration, viscosity of the broth etc.). These properties vary not only with the culture strain, but also during fermentation. A second reason is that gas hold-up is rarely important and seldom measured in laboratory-scale fermentations where most of the research is done. (Laboratory fermentors are normally not completely filled, so the level of the aerated broth may vary.)

Therefore, this article will not give any data on gas hold-up in fermentation media. Rather, it will describe how to measure the gas fraction in a particular process.

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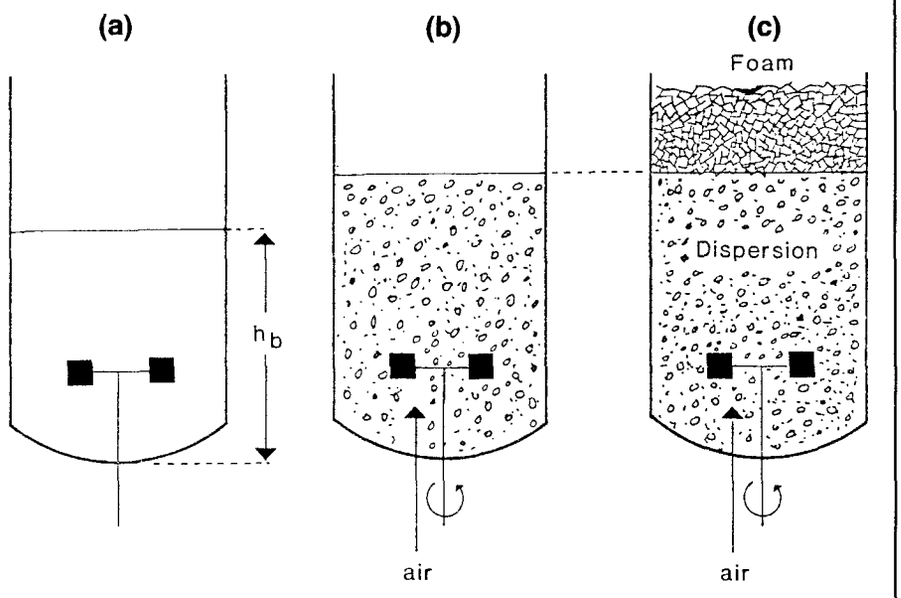
Defining gas hold-up

Gas hold-up can be measured as the ratio of gas volume to total

dispersion volume. This can be applied to determine either the overall hold-up or the local hold-up within a particular region of the fermentor. Local hold-up can also be measured as a ratio of times; the time that a given point is in the gas phase is compared to the total time. These two ways of defining the gas hold-up are different and have their own specific applications. The overall hold-up determines the broth weight charge per fermentor. The local gas hold-up is used for optimization studies on mass transfer. It is important to know how much gas is present in the fermentor zone where the oxygen transfer is high, e.g. in the stirring zone[1]. A high gas hold-up, especially in the stirrer zone, will increase the oxygen transfer rate.

Theoretically, local and overall hold-ups can be compared to each other by determining the average local hold-up. Especially when there are large hold-up gradients, this will require many different measurements of the local hold-up[2]. This comparison also can be used for verifying a particular measurement technique.

Fig. 1



Measuring overall gas hold-up by broth surface height. (a) Broth height (h_b) in unaerated broth; (b) increased overall volume due to gas hold-up (liquid volume is the same as (a)); (c) foam layer on aerated broth (which does not contribute to gas hold-up).

Total average gas hold-up

For determining the average volume fraction of the gas in the fermentor, both gas and liquid volumes must be measured. The volume of the gas in the fermentor is the difference between the measured volume of the gas–liquid two phase dispersion (V_{disp}) and the volume of the broth (V_b). The gas hold-up ϵ_G is given by:

$$\epsilon_G = \frac{V_{disp} - V_b}{V_{disp}} \qquad (1)$$

To measure the total volume of the dispersion (V_{disp}), the following methods can be used.

The position of the surface of the aerated broth can be measured on a calibrated scale on the wall of the fermentor. This is easy in a transparent fermentor. In stainless steel production fermentors, pieces of steel can be welded at certain heights to determine the height of the broth. This method can run under sterile conditions, but it is not very accurate because of the fluctuating broth surface[1].

Capacitive sensors are used to determine the dispersion height[3]. Under highly turbulent conditions, this method can cause problems because drops of liquid have influence on the measured capacitance. In mycelial fermentations this method cannot be used because of growth on the surface of the sensor.

The level of the dispersion can be kept constant by using an overflow to remove broth (Fig. 2)[4]. One problem with this is that the broth must be recycled because the surface turbulence will increase the overflow and lower the broth level[5]. This method cannot be used under sterile conditions because of the pumps and piping required.

Other methods are used for determining the volume of the broth (V_b).

A pressure cell can measure the pressure difference between the bottom and the top of the fermentor. Since the pressure difference is caused by the hydrostatic pressure of the broth, the broth weight can be determined. The broth weight is divided by its density to give the broth volume. Particular attention should be paid to the influence of the stirrer speed on the pressures that are measured.

Calibrated height measurements can be taken before air is blown into a fermentor. During a fermentation, however, this method cannot be used because the organisms constantly need oxygen, or produce other gases or, in fed-batch fermentations, because the broth weight changes. The broth weight can be calculated by a mass balance over the fermentation.

Some modern fermentors are equipped with piezo-electric devices for continuous weight measurements. This is probably the best way to determine the weight of the broth.

The measurement of the weight (or volume) of the broth must be very accurate. Any inaccuracy in the broth volume can result in large errors in calculating the gas hold-up (Eqn 1).

Local volumetric gas hold-up measurements

The gas hold-up in a particular region of a fermentor can be measured as a volume fraction or as a time fraction.

Many techniques have been developed for determining the local volume fraction of gas in a part of reaction vessels. Not all of these can be used in fermentors. For a detailed description of all these techniques, see literature cited in the reference list.

One method is to determine the mean local density of the dispersion by measuring the static pressure difference between two points of known vertical distance. This is widely used in bubble column fermentors[2,6–8]. The pressure difference ($\Delta\rho$) between the two points is equal to the hydrostatic pressure:

$$\Delta\rho = \rho_{disp} \cdot g \cdot \Delta h \qquad (2)$$

where ρ_{disp} is the density of the dispersion, g the gravitational acceleration and Δh the height between points. Since

$$\rho_{disp} = (1 - \epsilon)\rho_l + \epsilon\rho_g \quad (3)$$

where ρ_l is the density of the liquid and ρ_g the density of the gas, the gas hold-up is given (combining Eqns 2 and 3 and neglecting the density of the gas) by

$$\epsilon = 1 - \frac{\Delta\rho}{\rho_l g h} \quad (4)$$

Although manometers are used in almost all the published examples for measuring the pressure difference, these cannot be applied aseptically. In fermentation broth under sterile conditions, special pressure sensors can be used. These are also far more accurate than ordinary manometers.

A second possible volume-averaged local gas hold-up determination involves measuring the electric conductivity of the two-phase dispersion. This method cannot be used in fermentation broths, however, because the electric conductivity of a dispersion depends not only on the gas fraction but also on the concentration of salts. Salt concentrations change constantly during a fermentation.

Adsorption of radiation (radioactive and microwaves) is another possibility. Many different methods have been used, all based on the principle that liquids absorb the radiation much better than gas. These methods are applicable for hold-up measurements in fermentation broths. A detailed discussion on the various techniques is given by Hewitt[9].

However, even though measurement of the velocity of sound in a dispersion can be used to determine the gas hold-up, it is unsuitable for fermentation media. The reason is simply that the sound velocity in a fermentation broth does not vary significantly within the range of likely gas hold-up values (5–25%)[10].

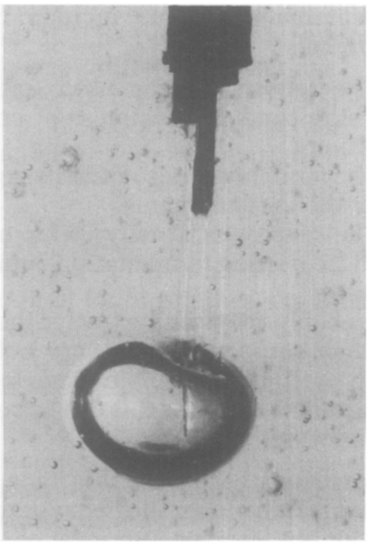
Local gas hold-up (time averaged)

In recent decades, a lot of research has been directed at developing sensors to determine the gas fraction by measuring the time that a certain point is in the gas or liquid phase. The local gas hold-up is given by dividing the time that the point contained air by the total time of the measurements. All systems for these kinds of measurements have a lot in common and a number of general remarks can be made about them.

- The sensors are all small and fragile; they must be small relative to the smallest bubbles in the fermentor.
- Since the signals are very fast (a bubble only takes a few milliseconds to pass the sensor), advanced electronics is required.
- Measurement of the time that the sensor is inside a bubble has to be very precise. Therefore, the response time of the sensor has to be very small (10 μs)[11].
- The sensor can influence the bubble in several ways[2]. These may cause serious errors. Firstly, the bubble can be deformed by the sensor (Fig. 3). Therefore, local hold-up sensors will not operate correctly in very viscous broths. Secondly, the bubble can be centred around the sensor because of interfacial tension effects. This can result in a higher residence time in the region of the sensor than would have otherwise been the case. The third important source of errors is the deflection of bubbles. The sensor may touch small bubbles and deflect them without entering them. To avoid this, the sensor should be very small compared to the bubble size. Measuring in non-coalescing media (e.g., those rich in protein) will be particularly affected because very small bubbles are present in these media.

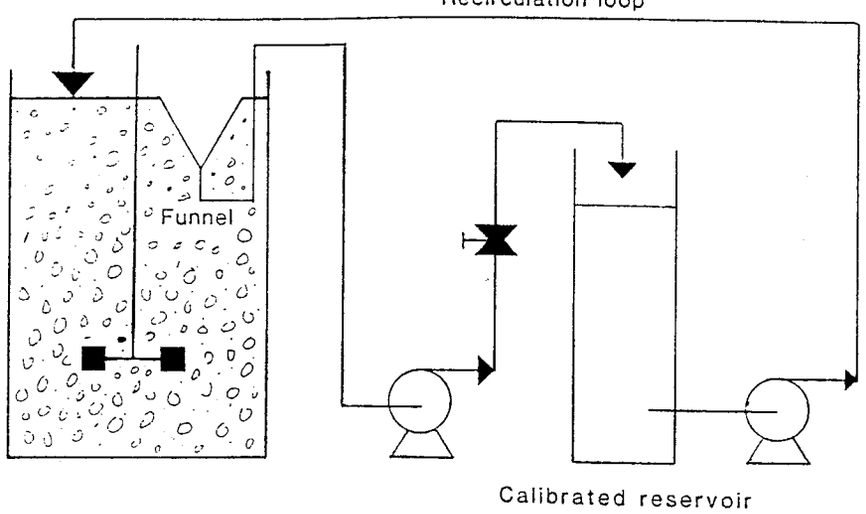
This is a long list of disadvantages of this type of sensor. However, these sensors can give other information on the gas–liquid dispersion besides that on gas hold-up. Advantages of

Fig. 3



Influence on the bubble shape of a local gas hold-up sensor in a viscous fluid. The bubble shown is in a carboxymethylcellulose solution (viscosity, 150 mPa s).

Fig. 2



The equipment for determining the height of the broth.

these sensors in relation to other measurement systems include the following:

- The sensors can be used under sterile and explosion-safe conditions.
- The fermentation is not influenced by the measurements.
- The sensors can easily be mounted in existing fermentation equipment.
- Probably most importantly, these small sensors can measure hold-up profiles and bubble diameters. In bubble columns this can be very important[2,11].

However, the use of this type of sensor is rather complicated. For this reason, but also because most of the sensors are not commercially available, they are not expected to be used in the near future.

Individual sensor techniques

The oldest technique for determining the time averaged local gas hold-up is based on measuring the difference in electric conductivity between the liquid phase and the gas phase. The main advantage of this technique is that very small sensors can be produced. However, these have relatively high response times. This may cause significant errors when the velocity of the bubbles is high or when the bubbles are small.

Another technique is based on measuring the difference of heat conductivity between the liquid phase and the gas phase. A commercially available standard hot-film anemometer probe can be used for this purpose[12]. This method is influenced by bubble deflection and delayed penetration of the sensor and is, therefore, unsuitable for fermentation measurements.

A relatively new technique is to measure the difference in the refractive index between gas and liquid[13]. The method can be realized relatively simply using glass fibers and opto-electronic couplings normally used in telecommunication[14]. This method can be applied in sterile process conditions. Very accurate measurements are possible because of the fast response time of the sensor. The liquid hold-up in a liquid dispersion can also be measured, as long as there is enough difference in refractive index between the two liquids. This could be useful for research into recovery processes; for instance, in determining the hold-up of an organic phase in an extraction process.

The last method of determining the local gas hold-up is by extracting the dispersion at a certain point by suction. The main problem of this technique is that 'iso-kinetic' sampling is required. This means that the movement of the dispersion during suction must have exactly the same speed as the undisturbed flow would have had. Since it is not possible to realize this condition in a fermentor, the method can lead to serious errors and should, therefore, not be used.

Conclusions

The gas hold-up is a very important parameter in biotechnological processes because it influences the mass transfer and the productivity of a fermentor. Since the gas hold-up is strongly dependent on the properties of the broth, no correlations are available to be used in predicting the hold-up under given fermentation conditions. Therefore, gas hold-up must be measured.

Before measuring the gas hold-up, it is necessary to consider what exactly has to be measured (local or average hold-up). An appropriate method can then be chosen. Since every method has its own applications and difficulties, measuring gas hold-up is never simple. Attention must be paid to the possible sources of error.

When measurements are performed correctly, however, a knowledge of gas hold-up will yield considerable information about a biotechnological process and its possible optimization.

References

1 Oosterhuis, N. M. G. (1984) *Scale up of bioreactors – a scale down approach* Ph.D. Thesis, University of Delft
2 Van der Lans, R. J. G. M. (1985) *Hydrodynamics of a bubble column loop reactor* Ph.D. Thesis, University of Delft
3 Cimorelli, L. and Evangalista, R. (1967) *Int. J. Heat Mass Transfer* 10, 277–288
4 Achwal, S. K. and Stepanek, J. B. (1975) *Chem. Eng. Sci.* 30, 1443–1444
5 Warmoeskerken, M. M. C. G. (1986) *Gas-liquid dispersing characteristics of turbine agitators* Ph.D. Thesis, University of Delft
6 Guy, C., Carreau, P. J. and Paris, J. (1986) *Can. J. Chem. Eng.* 64, 23–35
7 Verlaan, P., Tramper, J. and Van't Riet, K. (1986) *Chem. Eng. J.* 33, 1343–1353
8 Siegel, M. H., Merchuck, J. C. and Schugerl, K. (1986) *Am. Inst. Chem. Eng. J.* 32, 1585–1596
9 Hewitt, G. F. (1978) *Measurements of two phase flow parameters*, Academic
10 Van Wijngaarden, L. (1972) *Annu. Rev. Fluid Mech.* 4, 369
11 Zuber, N. and Findlay, J. A. (1965) *J. Heat Transfer* 87, 453
12 Wang, S. K., Lee, S. J., Jones, O. C. and Lahey, R. T. (1984) *J. Am. Soc. Mech. Eng.* 31, 39–45
13 Burgess, J. M. and Calderbank, P. H. (1975) *Chem. Eng. Sci.* 30, 743–750 and 1107–1121
14 Frijlink, J. J. (1987) *Physical aspects of gassed suspension reactors.* Ph.D. Thesis, University of Delft

Erratum

Regulatory requirements for licensing medicinal products of biotechnology, by Mary E. Duncan, Frances A. Charlesworth and J. P. Griffin (1987) *Trends in Biotechnology* 5 (12), 325–328.
An editorial error led to the impression that Mary E. Duncan was at The Association of the British Pharmaceutical Industry. This is not so. Mary Duncan is an independent consultant on registration of biological and biotechnological products at 15 Tedworth Square, London SW3 3DR, UK. We apologize for this error.