0273–2289/87/1403–0207$02.60

Simple Measuring Techniques for the Determination of Bubble- and Bulk-Phase Velocities in Bioreactors

A. LÜBBERT,* T. KORTE, AND B. LARSON

Institut für Technische Chemie, Universität Hannover, D-3000 Hannover, Germany

Received and Accepted August 14, 1986

ABSTRACT

Ultrasound Doppler pulse velocimetry for the measurement of bubble velocities and local temperature pulse time-of-flow techniques for determining the flow velocities of the liquid phase are two complementary new measuring techniques to gain information on the fluid dynamic behavior of bioreactors. Both methods show considerable advantages over conventional techniques. They proved to provide reliable results during biotechnological cultivations. Results from runs in airlift loop bioreactors are reported.

Index Entries: Bulk-phase velocities, measurement of in bioreactors; bubble-phase velocities, measurement of in bioreactors; bioreactors; ultrasound Doppler pulse velocimetry; flow velocities; fluid dynamic behavior; airlift loop bioreactors.

INTRODUCTION

Local flow velocities of the different phases are the central physical properties characterizing the fluid dynamics in multiphase systems of chemical or biochemical reactors. During real fermentations they are difficult to measure because most fermentation media are unfriendly environments to the sensor elements. Furthermore, strong interactions between the contributions of the motions of the different phases on the measuring signals impede an extraction of the information one is interested in.

Most methods, although well-established in simple model media, fail in sufficiently aerated cultivation broths of aerobic fermentations. Unfortunately, the elegant modern optical methods cannot be applied because most fermentation broths are optically opaque, and the microorganisms grow on the windows, so that a light transmission is prevented after the layer becomes too thick. Therefore, one must rely on probe techniques in most practical cases.

Wall growth is also a severe problem for the techniques in which probes must be introduced into the cultivation broth. But there are some possibilities to reduce the primary problem of intensity loss related to wall growth. The local temperature pulse time-of-flow techniques for determining the flow velocities of the liquid phase, which we describe in this contribution, gain their information from the runtime of temperature pulses rather than from simple temperature measurements.

Another difficulty is that probes do disturb the flow, at least on the scale of the probe diameter. This is especially essential for the measurement of velocities of small bubbles because small bubbles experience small buoyancy forces and have, therefore, small relative velocities as compared to the liquid in which they rise. Thus, they follow the liquid phase in turning aside the sensor. Therefore, one is interested in a method in which the fluid flow at the measuring volume is not disturbed by a probe. In this article we introduce a new ultrasound Doppler pulse velocimeter for measuring local distributions of bubble velocities in bioreactors.

TEMPERATURE PULSE TIME-OF-FLOW TECHNIQUE

General Description

The local temperature pulse time-of-flow technique for determining the flow velocities of the liquid phase in bioreactors is a tracer method. Instead of a material tracer we used heat for marking the liquid-phase fluid elements. This is accomplished by a small heating wire. At some adjacent place downstream, a temperature detector, e.g., a resistancy thermometer, senses the labeled fluid elements. From the time-of-flow of the fluid elements and the known distance between heater and sensor, one can calculate a good estimate of the liquid-phase flow velocity.

Both the heater and the temperature sensor are built up in the same way as hot wire anemometer probes, thereby using the long-time experience accumulated in the construction of hot-wire anemometers concerning minimal disturbances of the flow by the probes. The heating element is a platinum wire of 0.075 mm diameter at a length of 4 mm suspended between the tips of two thin prongs, as shown in Fig. 1, to which they are welded, as is known from hot-wire anemometers. The detector was made in the same way, its wire has a smaller diameter of 0.01 mm.

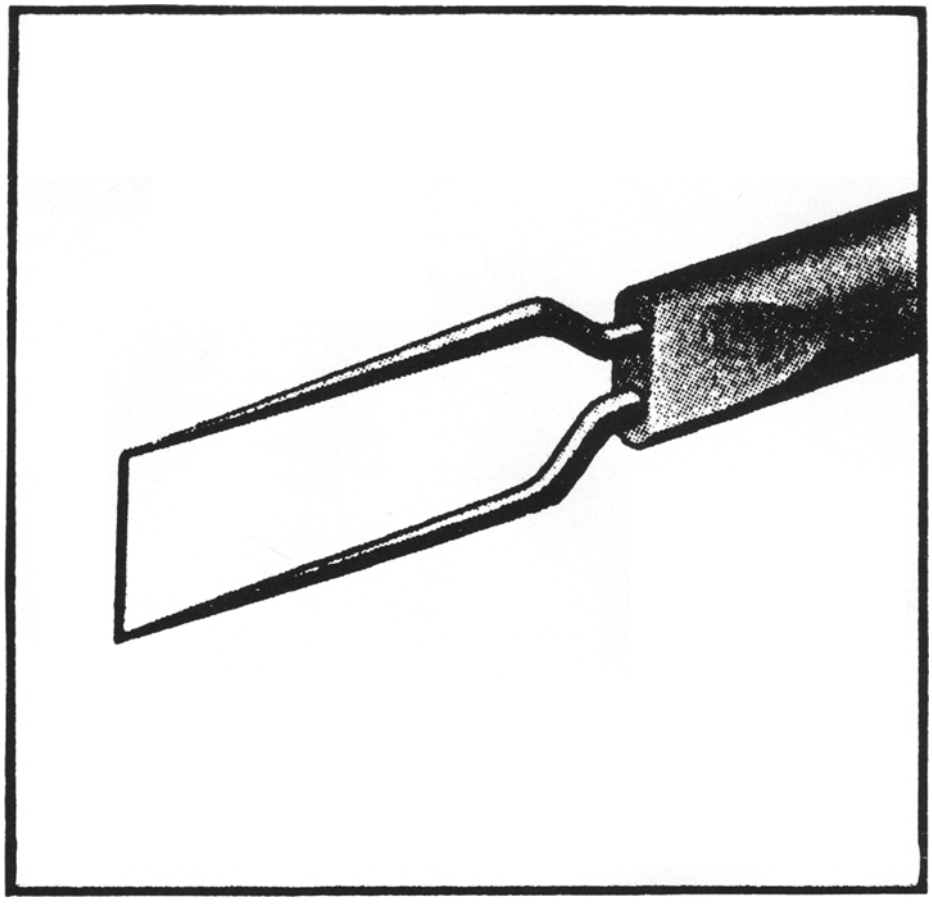

Fig. 1. Probe used as heating element in the pseudorandom local temperature pulse time-of-flow measurement.

As compared to hot film anemometers, the temperature pulse technique has significant advantages: (1) The information is extracted from the time-delay of temperature pulses instead of the amount of heat flow from a heated surface into the fluid. The latter is significantly changed when wall growth appears. (2) The disadvantage of a point-like measuring volume is circumvented. Point-like measuring volumes in gas-liquid two-phase-flows, as in hot wire anemometers, suffer from the fact that the velocity signal displays invalid information every time the probe is inside a gas bubble. If it is possible at all, a safe detection of these parts in the signal and their elimination takes much computing time, so that no real-time measurements during a biotechnological cultivation are possible. (3) The hardware is much cheaper, so that it is possible to install the device at every bioreactor. This is because both the probes and the pertinent measuring electronics are much cheaper than those for hot-film anemometers. These advantages are gained, however, at the cost of less information on the frequency content of the velocity fluctuations.

Pseudorandom Pulse Technique

Dirac pulses are the normal way for tracer injections into a flow. In bioreactors, however, the use of these signal forms has the disadvantage that very large heat pulses must be introduced to obtain acceptable responses at the detector. Such high-heat inputs have two significant disadvantages: (1) the high temperatures destroy the cells and build a thick layer on the wire surface; and (2) the liquid phase behaves in a different way at higher temperatures, leading to a systematic error.

Instead of Dirac pulses, we used a pseudorandom sequence of rectangular pulses, as shown in Fig. 2. By using pseudorandom input signals, it is possible to enhance the signal-to-noise ratio of the resulting time-of-flow distribution by more than one order of magnitude, as compared to signal Dirac pulses. At predetermined measuring times, this permits reduction of the temperatures at the heating elements drastically.

Because of the pseudorandom sequence of input pulses, one obtains a system response that is not the time-of-flow distribution as such, by using Dirac pulses. Instead, the distribution must be recovered from the known input signal and the measured system response as detected at the temperature sensor.

A cross-correlation of the measured signal with the known input signal leads to the resultant time-of-flow curve. It can be shown that for the method to work it is necessary for the auto-correlation function of the input signal to be a Dirac function (*1*). This is given for pseudorandom signals.

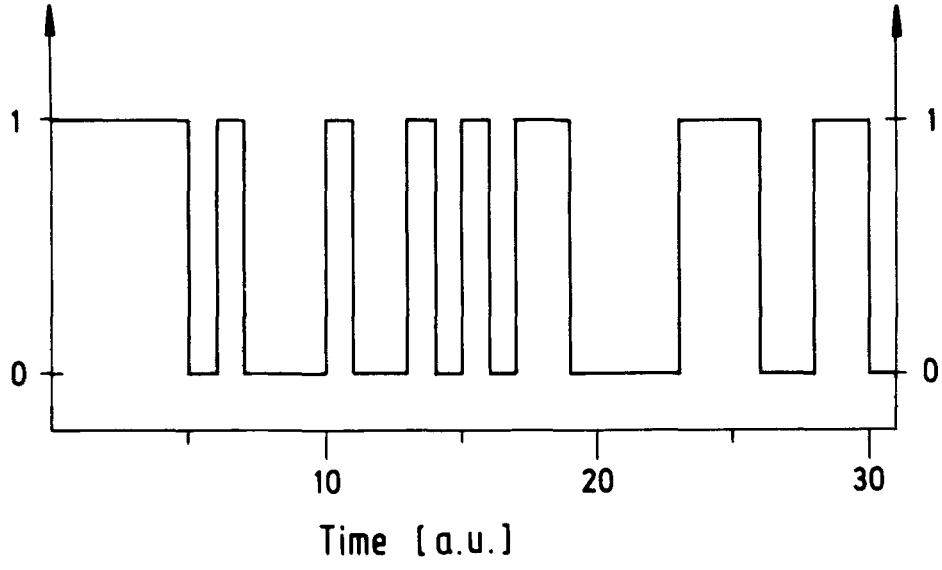

Fig. 2. Pseudorandom signal, which can be used as an input signal for the temperature pulse method to obtain time-of-flow distributions. The binary signal is divided 31 equidistant time steps. Its autocorrelation function is a technical Dirac pulse.

Analysis Hardware

Usually the calculation of correlations requires a computer. In this case, however, the necessary computations could be reduced to such a level that it is possible to run the complete automatization of the measuring system on a single, extremely simple eight-bit microprocessor system.

The necessary components are summarized in Fig. 3. The central component is a Motorola 6802 microprocessor. By a digital output integrated circuit (IC) module, the system controls the pseudorandom heating signal, and by an analog-to-digital converter IC, it reads the temperature signal from the temperature-sensing element. Quasiparallel to these activities, the system calculates the cross-correlation function, takes an ensemble average over successive correlation records, and displays the actual ensemble average via a digital-to-analog IC onto an oscilloscope. The control program is stored on an Eprom-IC, which is not shown in the figure.

The system can be connected to a larger computer via a serial output IC. In this experiment a connection to a personal computer DEC PC350 was installed. On this larger computer it was possible to fit a model equation, e.g., the axial dispersion model, to the data.

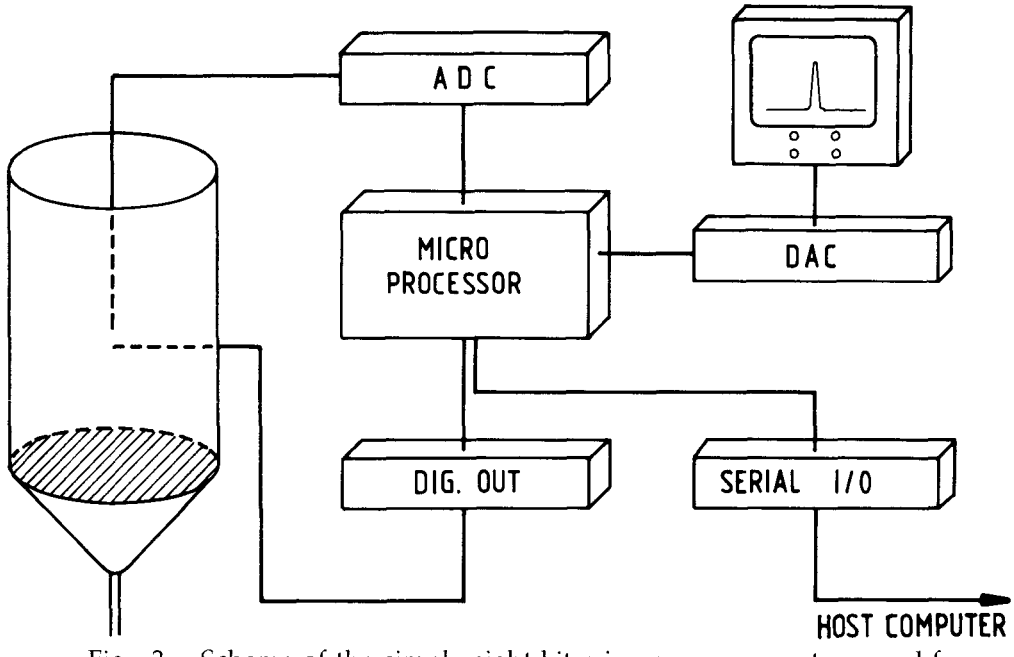

Fig. 3. Scheme of the simple eight-bit microprocessor system used for a complete automatization of the measuring system. The central component is a Motorola 6802 microprocessor. By a digital output IC the system controls the pseudorandom heating signal, by an adc IC it reads the temperature signal, actual results can be displayed via a dac IC onto an oscilloscope. The serial interface IC is used for a communication with a host computer.

All components can be housed on a single printed circuit of standard European format.

Experimental Difficulties

Problems with the method arise in three fields: errors related to a delayed heating of the fluid elements; electrical couplings from the heating element onto the sensor; and building of an insulating layer on the heating element.

Because the fluid elements around the heating wire cannot be heated as fast as the control signal changes, there is a time delay between the electrical control signal that is used in the correlation calculation and the actual temperature of the fluid elements. This leads to a systematic error in the time-of-flow, which can be eliminated only by making two measurements with different distances between heater and sensor.

Electrical coupling effects can be divided into three cases: conductivity over the conductive fermentation broth; inductive coupling, and capacitive coupling. Conductive coupling can be avoided by simply insulating one of the probes. Inductive coupling proved to be important only at small distances between sensor and heater (less than 1 mm). Such small distances are not used because these are in the scale of these devices.

Troubles because of capacitive effects remain the only important coupling effects. This error can be eliminated by changing the direction of the heating current every successive sweep (2).

Typical Results

Figure 4 displays a typical time-of-flow distribution obtained with this device during a yeast fermentation in a concentric airlift loop reactor on the axis of the inner tube, the riser. Detector and heater positions were on the axis 5 mm apart. The input signal consisted of 31 equidistant time-slice elements, at which the current for the heater was switched on or off pseudorandomly. Simultaneously, at every time-slice element, 2 measuring values were taken from the sensor. From both, a mean value was calculated, resulting in a record of 31 mean measuring values per cycle. Such sweeps of 31 elements were cross-correlated. The final result was obtained as an ensemble average over 5000 sweeps.

After measuring all sweeps, the resulting time-of-flow curve was transferred onto the personal computer to fit an axial dispersion model to the data. The result of the fit is also plotted into Fig. 4. From this fit characteristic numbers of the time-of-flow curves are extracted: the mean flow time and a characteristic number of the width of the distribution, a so called local Bodenstein number.

The reliability of the measured results for these characteristics are dependent on the gas throughput in the gas/liquid reactor, as are all other measuring techniques. But, as opposed to other techniques the re-

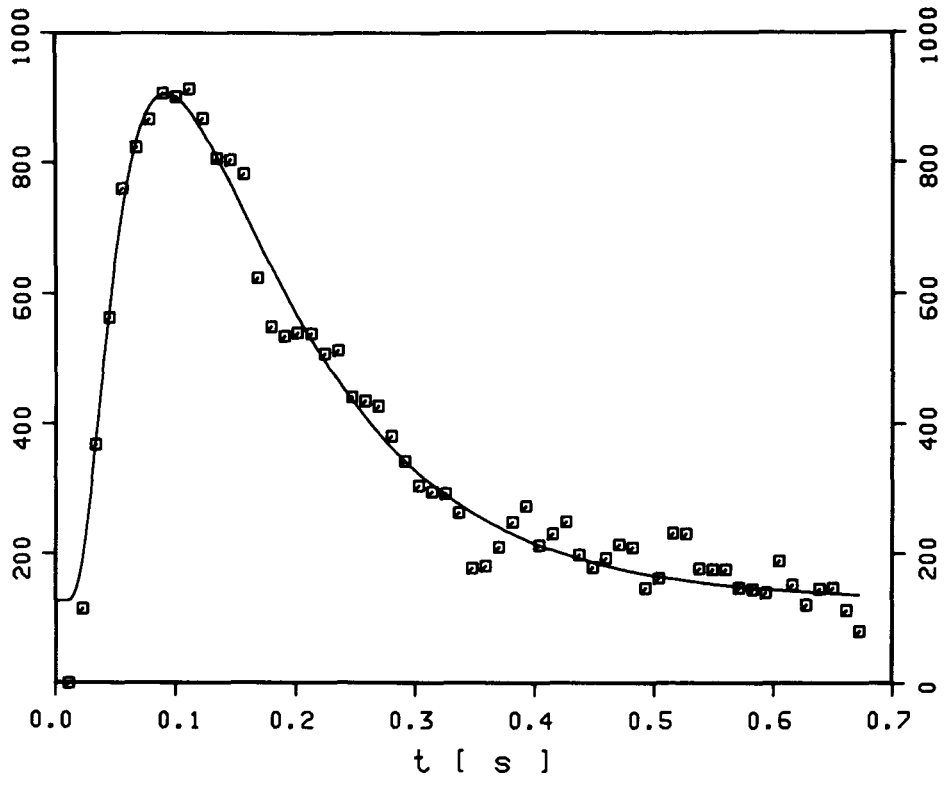

Fig. 4. Typical time-of-flow result as displayed by the microprocessor unit (symbols), together with the nonlinear fit to an axial dispersion model, as calculated on the attached personal computer (continuous line). From the fit characteristic numbers of the time-of-flow curves are extracted: The mean flow time and a characteristic number of the width of the distribution, a so called local Bodenstein number.

sults improve with increasing gas rates. This is demonstrated in Table 1, which comprises the resulting characteristic numbers together with their mean deviations as a function of the superficial gas velocity. Therefore, the method is predestined for measurements at higher gas throughputs, where other methods fail.

TABLE 1
Results from Measurements with the Local Temperature Pulse Time-of-Flow Method Taken at Different Gas Throughput[a]

W_{SG},CM/S	T_M,MS	DT_M,%	*Bo*	*DBo*,%
1.08	62.9	8.6	4.7	20.0
2.17	42.38	3.87	6.4	13.9
3.25	33.42	2.15	6.9	11.7
4.33	26.76	1.95	7.7	6.5
5.41	22.91	1.64	7.9	6.5

[a]For five different superficial gas velocities, the mean flow time and the local Bodenstein number is printed together with their relative errors.

ULTRASOUND DOPPLER PULSE VELOCIMETRY

General Information

Ultrasound Doppler pulse velocimetry is a new variation of the ultrasound Doppler velocimetry (3) for the measurement of the bubble velocities in the cultivation broths of bioreactors.

The general principle of the method is displayed in Fig. 5. Ultrasound is emitted from a transmitter into the dispersion. Since gas bubbles are excellent reflectors for ultrasound, part of the power is led into the detector. As compared to bubbles, reflections at microorganisms suspended in the cultivation broths can be neglected. From the difference in frequency between the transmitted and the received signal, the bubble

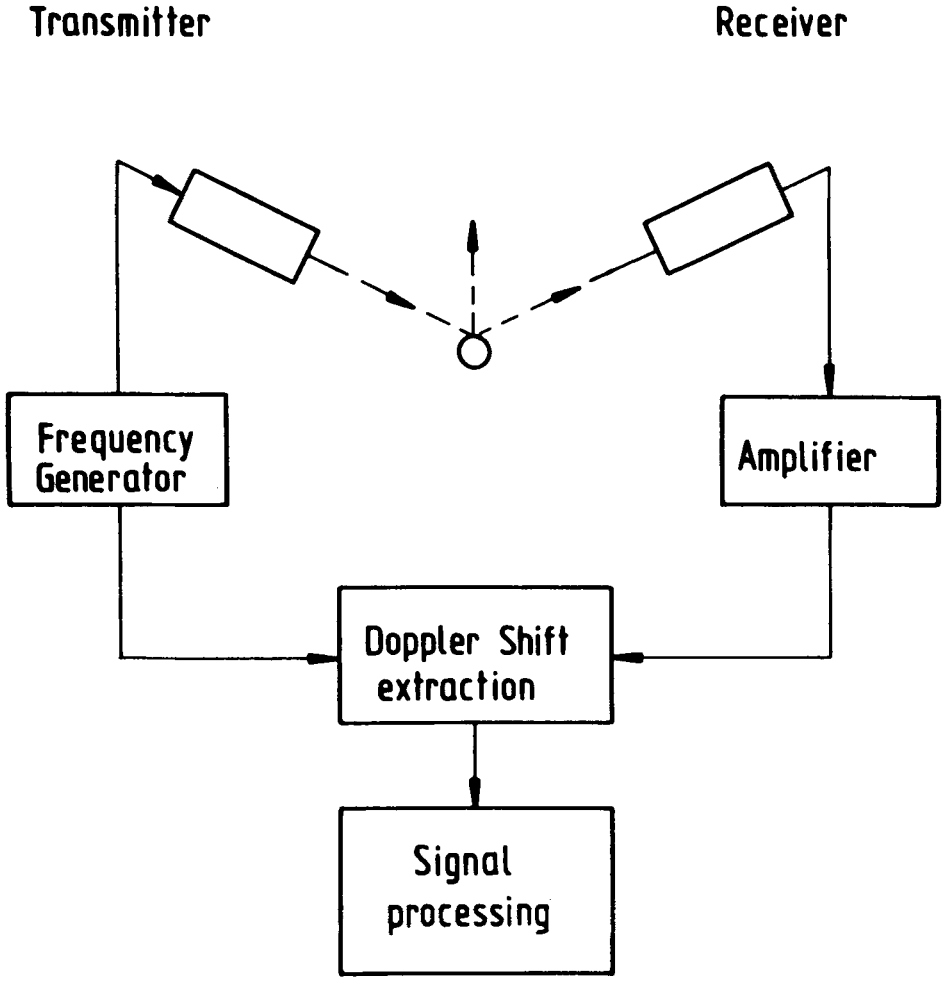

Fig. 5. Scheme of the general principle of the ultrasound pulse Doppler method. An ultrasound beam is emitted from a transmitter into the dispersion, reflected at a gas bubble, and registrated at a detector transducer. From the exciting signal and the registered signal, the Doppler shift is extracted and fed into an analysis unit.

velocity can be calculated according to Doppler's principle. The Doppler shift is proportional to the velocity component of the bubble directed along the bisector of the angle between the incident ultrasound wave and the reflected beam, as indicated in the figure.

Because of the complicated beam characteristics of the transducers and to suppress the influence of multiple reflections, it proved to be better to transmit ultrasound pulses instead of a continuous beam. This offers the possibility to further limit the measuring volume to the region of intersection of the main "beams" of the transmitter and receiver transducer by appending a run-time criterion to the usual geometrical restrictions.

Ultrasound Transmitters and Detectors

Equivalent devices can be used for the ultrasound transmitter and the detector. They are based on piezoelectric ceramic slices, excited by an electrical frequency generator.

The ultrasound beam characteristics can be influenced by the choice of an appropriate working frequency (*4*). Adequate measurements of bubble velocities in bioreactors can be done at frequencies between 1 and 10 MHz.

Applications in bioreactors require the sensors to be sterilizable. Unfortunately, the probes that are on the market do not fulfill this condition. Therefore, special probes had to be constructed.

Transmitter and receiver have been built up from cylindrical piezoelectric ceramic plates of 10 mm diameter, at a height of 0.5 mm, for a working frequency of 4 MHz. Both plane end surfaces are covered with a nickel layer, to which the power supply lines are soldered. At the back of the plate, the feeding lines are cast in epoxy resin. The transmitter is supplied by a frequency generator at about 1-W output power.

These simple probes proved to be sterilizable at temperatures of about 120°C. They also withstand the cultivation broths during an operation over several weeks.

Pulse Techniques

From the geometrical arrangement, one can calculate the mean propagation path of the ultrasound pulses. With the propagation velocity of about 1500 m/s in the biosuspension, one can calculate the mean run-time of the pulses from the transmitter to the detector. Only those pulses that arrive at the detector within a predefined time-window around the mean run time are registered and can contribute to the result.

The transmitter was fed with short wave packets of eight cycles from a 4-MHz sine wave. The pulse repetition rate was adjusted, to ensure that the pulses could be identified at the detector.

The volume of intersection of the main beams of the transducer characteristics is the second restriction on the measuring volume.

As checked out experimentally with chains of single bubbles rising in water during a test experiment, this technique gives much better results than using continuous waves.

Signal Processing

A real time analysis of the Doppler frequencies requires a high-performance digital computing device. We used an array processor attached to a standard PDP11 process computer (*5,6*).

The Doppler signal is extracted electronically from the transmitted and the detected radio frequency signals. Its bandwidth is about 10 kHz. To differentiate between positive and negative frequency shifts according to positive and negative bubble velocities, we used an electronical quadrature detection technique, which yielded two signals from the receiver shifted in the phase by 90°.

These two signals were digitized continuously by means of an appropriate ADC-interface at the array processor. From the two sample record of 512 signal values each, cross-spectral density functions were calculated by Fast Fourier Transformation (FFT) techniques. To smooth the spectral estimate, an ensemble average was taken over successive records of these cross-spectra (*7*).

Generally, these cross-spectra are records of complex values. From the imaginary parts, a good estimation of the mean bubble velocity can be calculated (*8*).

Estimation of the Specific Interfacial Area

The ultrasound power that is reflected into the detector depends in a natural way on the total surface per measuring volume, which acts as reflector for the incident ultrasound beam. Therefore, this power must be correlated with the specific interfacial area, a, which is a central property of gas–liquid reactors.

In a simple model one can assume that the reflected power is proportional to the specific interfacial area, a. Because the ultrasound is attenuated on the way from the transmitter to the measuring volume and from there to the detector, an attenuation factor must be introduced. Assuming a to be nearly constant along the propagation path of the pulses, one can use an exponential decay of the ultrasound power, according to Stravs (*9*). These results can be combined in an equation for the received ultrasound signal energy, E:

$$E(a) = ca \exp(-al/4)$$

where c is a constant and l the length of the ultrasound path; c must be determined by experiments. Assuming that a is larger than $4/l$, one can associate with each mean signal energy value an estimation for the specific interfacial area, a, by using this model equation as a calibration curve.

Typical Results

Figure 6 displays a typical result for a distribution of the axial component of the bubble velocity, as obtained in the riser of a concentric airlift bioreactor. It is obtained after 80 h of fermentation at a superficial gas velocity of 6 cm/s. The ultrasound path length was 42 mm.

Several curves obtained at different times during the fermentation show that the bubble velocity distribution changes drastically during cultivation. Because of the metabolism of the cells, the chemical and physical properties of the medium change continuously. In consequence, the bubble volumes as well as their shapes and surface motions are not stationary. This fact has severe consequences for the agitation characteristics and the mass transfer.

The amount of such changes can be conceived from a record of the maxima of the observed bubble velocity distributions, which are plotted in Fig. 7 as a function of the fermentation time. At 50 h the gas throughput has been increased from a superficial gas velocity of 4 to 6 cm/s. All velocity data proved to be reproducible within at least 5%; the variations of the measured values are, however, more than 50%. This means that the hydrodynamics change drastically during the fermentation run.

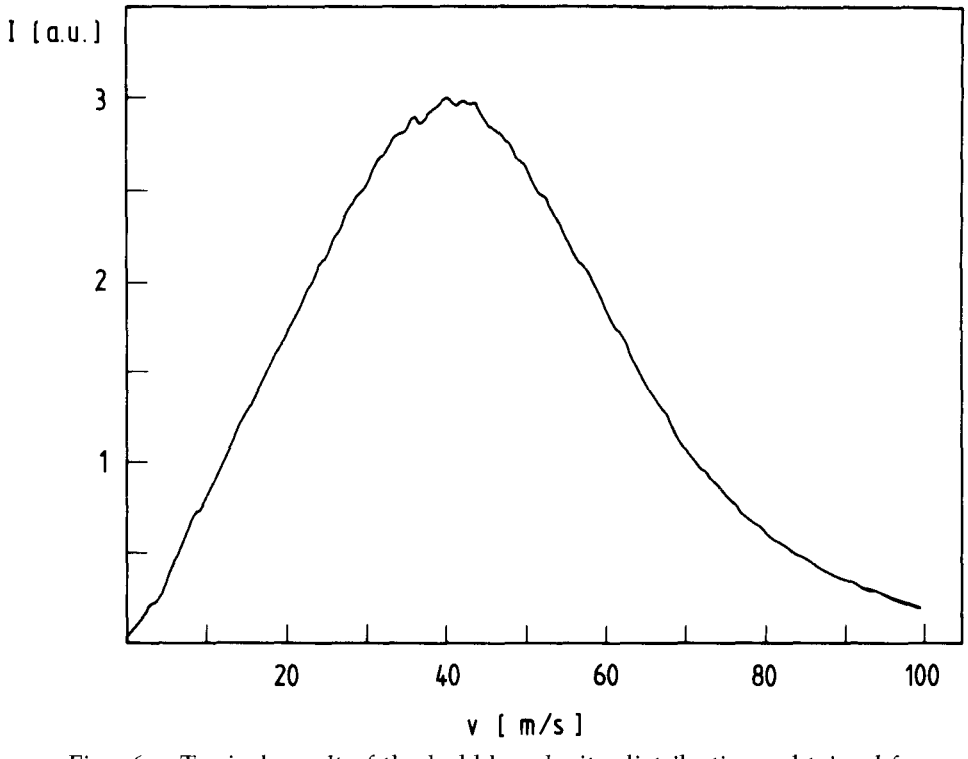

Fig. 6. Typical result of the bubble velocity distributions obtained from a fermentation in a concentric airloop bioreactor during a yeast fermentation. The curve is measured after 80 h of fermentation time. The measuring volume has been on the axis of the system, 80 cm above the gas distributor.

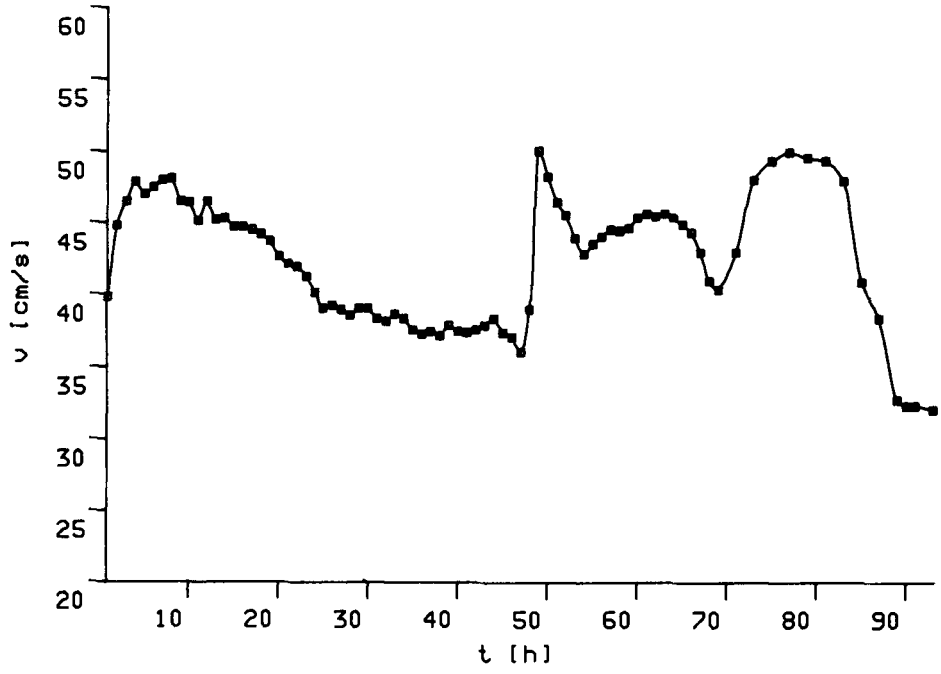

Fig. 7. Variation of the mean bubble velocity over the fermentation time. The values were taken at the axis of the tower-loop reactor, 80 cm above the gas distributor.

Much more variations are to be expected for the specific interfacial area, a, as estimated by the above argumentation from the mean ultrasound energy received at the detector. The result displayed in Fig. 8 was obtained during the same fermentation run as the data shown in Fig. 7. During the phase of exponential growth, which has been observed from about 10 to about 30 h, the specific interfacial area increases continuously by a factor of about 3.

Besides the measurement of bubble velocities, the ultrasound Doppler pulse velocimetry supplies, therefore, additional valuable information on fermentations.

ACKNOWLEDGEMENTS

The authors would like to thank the "Deutsche Forschungsgemeinschaft" for financial support. We also express our thanks to Professor Dr. K. Schügerl for his support of this work. This paper was part of the BHRA Conference held at Cambridge University; organized and sponsored by BHRA, The Fluid Engineering Centre, with the support of The Institution of Chemical Engineers and the Society of Chemical Industry.

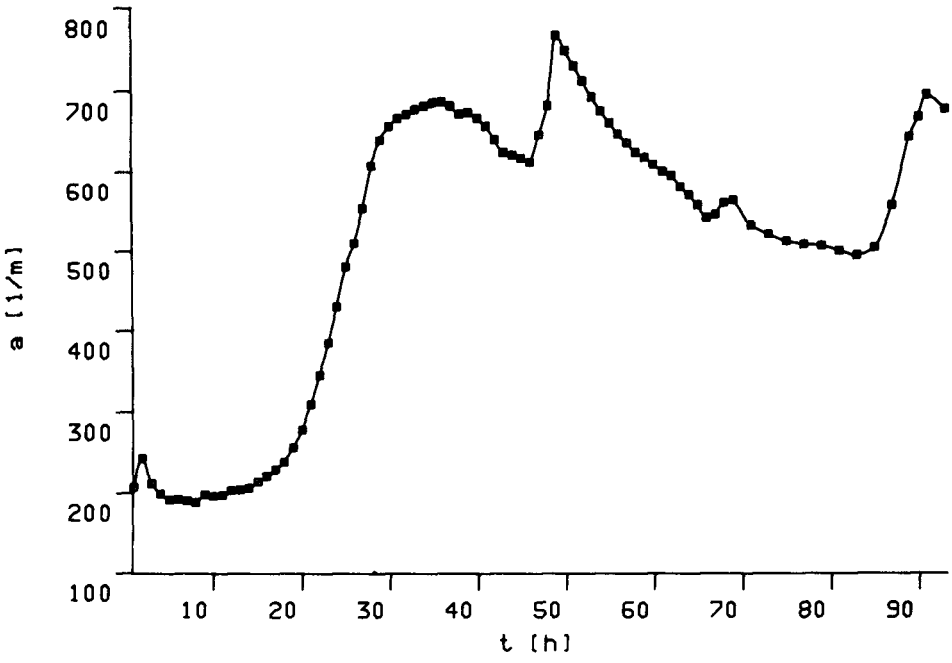

Fig. 8. Variation of the specific interfacial area, *a*, as estimated from the mean signal energy.

REFERENCES

1. Lübbert, A., Diekmann, J., and Rotzoll, G., (1982), in *Residence Time Distribution Theory in Chemical Engineering*, Pethö, A., and Noble, R. D., eds., Verlag Chemie, Weinheim, FRG, pp. 223–227.
2. Larson, B., (1985), *Time-of-Flow Measurements in Bubbly Flows by Means of Pseudorandom Temperature-Pulse-Sequences" (Laufzeitmessungen in Blasenströmungen mit Pseudostochastischen Temperaturimpulsfolgen);* Diplom Thesis, University of Hannover (in German).
3. Lübbert, A., Korte, T., and Schügerl, K., (1983), in *Measuring Techniques in Gas-Liquid Two-Phase Flows*, IUTAM Symposium Nancy, France, Delhaye, J. M., and Cognet, G., eds., Springer, Berlin, pp. 479–494.
4. Krautkrämer, J., and Krautkrämer, H., (1975), *Material Testing With Ultrasound (Werkstoffprüfung mit Ultraschall)*, Springer Verlag, Berlin (in German).
5. Korte, T. and Lübbert, A., (1984), in *Parallel Computing 83*, Int. Conf. on Parallel Computing, Berlin, 1983, Feilmeier, M., Joubert, J., and Schendel, U., eds., Elsevier, Amsterdam, pp. 341–344.
6. Korte, T., and Lübbert, A., (1985), *Comp. Chem. Eng.* **9,** 19.
7. Bendat, J. S., and Piersol, A. G., (1971), *Random Data—Analysis and Measurement Procedures*, Wiley-Interscience, New York, NY.
8. Korte, T. (1986) *Measuring Techniques for the Investigation on the Hydrodynamics of Multiphase Flows in chemical reactors ("Messtechnische Möglichkeiten bei der Untersuchung der Hydrodynamik in den Mehrphasenstroemungen chemischer Reaktoren)*, ongoing dissertation, Universität Hannover (in German).
9. Stravs, A. A., and von Stockar, U., (1985), *Chem. Eng. Sci.* **40,** 1167.