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Biosensors in Process Control [and Discussion]

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Biosensors in process control

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Improvement in bioprocess control will require development of monitors for a wide range of cell-growth and downstream-process parameters. These requirements are examined in terms of the development and application of biosensor devices and physical measurement techniques. Acoustic, dielectric and laser light scattering techniques are discussed primarily as monitors and analysers for biomass parameters. Developments with biosensor devices are discussed in terms of their application in membrane sampling-flow analysis probes and the potential of more direct biosensing principles.

1. Introduction

Few biosensor devices or new analytical systems have been developed for reliable application in process control. Small, intermediate and plant-scale fermentation systems are being instrumented and automated by means of computer control systems. More recently, the automation of aspects of downstream processes (cell harvesting, homogenization and extraction, together with product purification procedures) is also being considered. Although feedback control parameters have become more accessible and useful, the transducers (sensors and actuators) used have not changed significantly. Consequently, computer control relies largely on automating manual operations by using standard sequence-control procedures and installing flexible scheduling of sequence- and feedback-control protocols within the overall process.

Monitoring and control of temperature and pressure is well established. However, recent developments in monitoring such parameters at multiple points by using single, fibre-optic, distributed sensing techniques (D. Payne, personal communication) appear much more suited to control requirements. Although flow sensors are also well developed, application and measurement-range problems continue to prevent installation of reliable fluid and gas control régimes, particularly those that would be required to establish physiological and biochemical control procedures based on potential biosensor and chemical sensor devices (Clarke *et al.* 1984, 1985).

Fermentation practice continues to use pH, dissolved gases (especially oxygen and carbon dioxide) and redox potential as control parameters at small and intermediate scale, whereas control at pilot and large scale is often more restricted. More recently, mass spectrometry has become established for monitoring various gases (e.g. oxygen, carbon dioxide, ammonia and nitrogen) and volatile constituents (e.g. methanol and ethanol) in both vapour and fluid phases (Lloyd *et al.* 1986). There are even fewer parameters routinely monitored in downstream processes: UV-absorption monitoring of elution profiles of column chromatography is the only prevalent practice.

Although several advantageous biochemical control régimes for fermentation processes have been identified, both their installation and the identification of others await suitable biosensor devices. The virtual absence of sensor devices for downstream processes in even more apparent, although the applications are often less demanding and the requirements for better control, to prevent loss of expensive preparations and to improve product preparation procedures, are perhaps more acute.

2. Measurement parameters required

There is considerable similarity between the sensor and analytical specificity requirements of bioprocess monitoring and other major applications of biosensors (clinical or medical, environmental, and food or beverage). There are needs to analyse cell population characteristics, including cellular and intracellular parameters, as well as the environment of the cell (in this case the growth medium). Many products are macromolecules (such as enzymes and proteins) and, both during fermentation and downstream processes, there are needs for general monitoring techniques (e.g. molecular size, charge and conformation) as well as specific ones (e.g. enzymic activity). Table 1 summarizes these general requirements.

Table 1. Parameters required to improve bioprocess monitoring and control

cellular
cell concentration and growth
culture contamination (by microorganisms & viruses)
cell type (population distribution analysis)
cell viability
cell infrastructure: changes in cell envelope
production of inclusion bodies
organelles
cytosol: key enzymes and metabolites
cell environment
inorganic ions: major cations (K^+, Na^+, Ca^{2+}, Mg^{2+}, NH_4^+)
: major anions (PO_4^-, NO_3^-, HCO_3^-)
nutrients (sugars, amino acids, vitamins)
products (e.g. excreted metabolites, antibiotics, hormones, antibodies)
biomacromolecular
molecular size
isoelectric point
mixture resolution (on the basis of charge or size)
conformation (shape, subunit composition and folding)
activity (enzymic or ligand binding)

Fermentation processes are largely modelled as bulk, macroscopic biomass and medium compartments on the basis of the assumption that the population is homogeneous. Within the biomass 'black box', the importance of developing monitors of 'microscopic' parameters (e.g. intracellular constituents or cellular characteristics) is becoming increasingly recognized, as our dynamic understanding of the biochemistry and physiology of these processes becomes more advanced. However, it is also well recognized that both microbial culture and cell culture populations are heterogeneous, particularly as the scale of the culture is increased. Consequently, in addition to the above macroscopic and microscopic parameters, techniques are

required to analyse cell types or individual cells within any population, on the basis of particular physical and/or biochemical characteristics. Indeed, these capabilities are a prerequisite of identifying culture contamination and understanding mixed-culture processes.

3. General approaches to the development of suitable monitors

Before discussing the particular capabilities of individual analytical and biosensor monitoring techniques, conclusions can be drawn about their potential application by considering the underlying nature of the various measurement principles involved and the constraints imposed by process control applications.

(*a*) *Approaches to development of sensor systems*

Although automated biochemical assay procedures are being used in some installations, their online application is fraught with difficulties (e.g. achieving aseptic sampling, the requirement for skilled technical support, assay interference, costs of failure, maintenance and reagents). Similarly, although there are some applications for single-test, disposable biosensor devices, such as those receiving considerable attention for clinical applications, continuously monitoring biosensor devices will have much greater impact. Continuous monitoring techniques can be usefully classified as being based on either physical measurement principles directly or true biosensor devices (including chemical sensors).

The specificity of physical measurement techniques is generally improved by spectroscopy (with either time- or frequency-domain techniques), which allows extraction of considerably more information, to cope with the measurement complexity and interferences of biological materials. Physical methods generally depend on the differential absorption and/or scattering of applied radiation by the biological material. The contribution of scattering to the signal is generally larger for wavelengths approaching the size of the particle. As the wavelength of the applied radiation increases beyond the size of the particles being analysed, the contribution of absorption or similar processes becomes greater, as does the scale of the underlying process being analysed (table 2).

Light scattering effectively depends on the refractive index difference between particle and medium, being measurable for particles as small as proteins, whereas absorption (in the visible and infrared band) is dependent on the electronic transitions and vibrations of molecules, respectively.

Magnetic transitions of molecules, as used in electron spin resonance (ESR) and nuclear resonance (NMR) spectrometry, occur at longer wavelengths. Dielectric absorption generally depends on molecular dipoles, such as water, at microwave frequencies, and ionic dipoles at lower frequencies, where the size and conformation of the particle itself become important in the underlying relaxation process. Similarly, acoustic methods depend on density and viscoelastic properties, whereas at the upper range of ultrasound (MHz), the contribution of scattering becomes more significant.

Although analysing electronic and magnetic transition processes has value for relatively pure samples of biological molecules, the size of the process being analysed is small compared to the scale and complexity of most biological materials. Therefore, spectra are complex and difficult to use, except where natural or synthetic probe molecules are available. In view of the fact that many of the parameters required are of larger scale than molecular processes, it is surprising

TABLE 2.

(*a*) *Spectral regions of biological interest*

frequency...		THz	GHz	MHz	kHz	Hz	mHz
timescale...			ps	μs	ms	s	
wavelength...	μm	mm	m	km			
spectrum...	UV vis. IR	microwave	RF	LF	VLF		

(*b*) *Transitions and their applications*

electronic
UV vis. IR spectroscopy

magnetic
resonance spectroscopy (ESR, NMR)

laser particle motion and size spectroscopy

dielectric
dielectric time- and frequency-domain spectroscopy

ultrasonic or acoustic
absorption and propagation analyses

electrochemical
electrode processes

The frequency, timescale and wavelength bands of various spectroscopic and spectrometric techniques are related to the underlying transitions and relaxation processes involved for electronic, magnetic, dielectric, viscoelastic and electrode processes. Laser light scattering is included as an example of the analysis of noise signals resulting from particle motion by correlation techniques.

that little use has been made of absorption and similar processes occurring at longer wavelengths (table 2). However, for radiation scattering, considerably more use can be made of shorter-wavelength techniques, such as laser light scattering, because the wavelengths are close to the size of biological particles and the wave properties of the radiation can be used to analyse the translational and radial motions of the particles. The potential of acoustic, dielectric and laser techniques will be discussed later (§5) for monitoring the concentration, size, shape, movement and more diagnostic characteristics of biological particles important both of fermentation as well as downstream processing.

By definition, biosensor and chemical sensor devices are potentially capable of monitoring the chemical and biochemical characteristics of bioprocesses. The majority of devices reported are for species of low molecular mass. The development of monitors for environmental parameters (i.e. biochemical parameters in the medium compartment) of fermentation processes has tended to be recognized as being most important for monitoring particular products excreted into the growth medium (antibiotics, extracellular enzymes, and so on). However, many fermentation products, such as enzymes, remain within the cell. The use of this type of process has been increased because of the development of molecular- and cell-cloning techniques, which allow animal and plant sources of products to be replaced with fermentation and cell-culture processes. Control of these processes by using product biosensor devices is impossible without previous cell-extraction or permeabilization procedures. However, particularly at the research and intermediate scale of fermentation, there is growing interest in monitoring inorganic (major cations and anions) and biochemical species (sugars, amino acids and staling products such as carboxylic acids) in the growth medium, and this provides limited

applications for some of the above biosensor devices for species of low molecular mass. Although the cell controls its metabolism, it is recognized that the modification of medium parameters can significantly affect productivity (see, for example, Clarke *et al.* 1982, 1985). The requirement for such control practices has become greater as more use is being made of eukaryotic cell-culture techniques, where physiological understanding is less developed. Similarly, although some fermentation technologists have not seen the need to monitor small biochemical species in the growth medium, applications have developed where maintaining optimum culture physiology is difficult (e.g. the trend towards higher biomass densities to increase productivity, overproduction of metabolites, and so on).

The monitoring of enzymes and proteins in downstream processes is likely to become a more important application of biosensor devices. Unfortunately, however, considerably less attention has been applied to their development (see §4). The relatively poor reliability of chemical sensor and biosensor devices presents difficult application problems. Although various approaches are available for minimizing these problems when using present biosensor devices (see §4*b*), the need to develop inherently more reliable devices is also discussed as a longer term solution (see §4).

(*b*) *Applications requirements of bioprocess monitors*

Most requirements are well recognized and similar to those of other continuous monitoring applications: high specificity to cope with complex biological materials, accompanied by low interference or the ability to correct for interferences; simple operation and maintenance; reliability over at least a few days, and preferably a month, without operator involvement. However, fermentation practice places greater emphasis on the development of robust, steam-sterilizable probes. These basic requirements appear to have precluded development of many sensor techniques for many years. However, it has recently become expeditious and more productive to view these requirements as the operational constraints of the fermentation probe, relaxing the requirements of the sensor or measurement incorporated within the probe. Various approaches have been taken towards protecting and isolating sensors and measurement elements within robust steam sterilizable probes, from non-invasive physical measurements to porous membrane-protected flow probes containing more fragile biosensor devices (figure 1).

True biosensor devices rely on interfacial properties (e.g. electrochemical or optoelectronic) between the device and the analyte-containing medium. Therefore, unlike physical measurements, non-invasive measurements (figure 1*a*) cannot be envisaged and the sensing surface must be in direct contact with the medium (figure 1*b*, *c*). Consequently, fouling and poisoning of sensing surfaces, together with interference, are the major problems of application. Similarly, although the notional specificity of most biosensor devices is high by virtue of their biological sensitization, interference both at the levels of the biosensitization and the transducing elements can also be high in complex biological media. For these reasons it is becoming recognized practice to protect such devices from fouling and, to some extent, from poisoning and interference, by flow- and membrane-sampling procedures (figure 1*d*–*g*).

Although some fermentation technologists would find research use for flow-sampling systems (where sensors are incorporated in a non-return flow line, to avoid the need for sterilization and allow routine calibration) (figure 1*f*), minimization of fouling would also require removal of particulate matter, for example by using a cross-flow filtration module (figure 1*g*). Better approaches involve encasing the biosensors behind microporous filters or dialysis membranes,

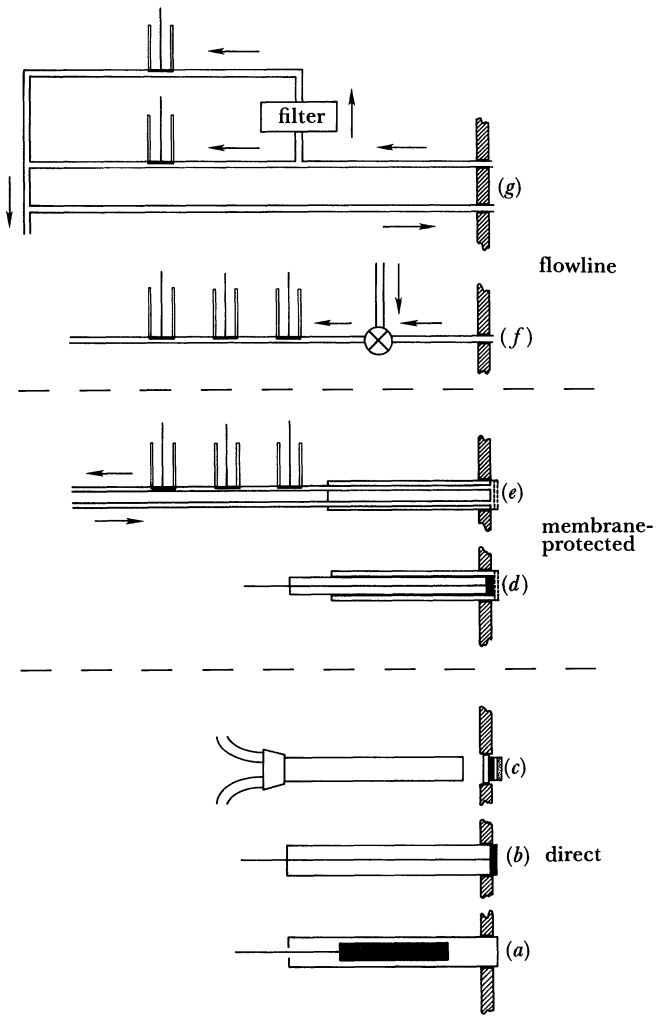

FIGURE 1. Developments in fermentation probes and continuous sampling techniques. (*a*) Directly and non-invasively monitoring probe applied *in situ*, where the sensor is wholly protected from fermentation broth. (*b*) Biosensor device directly exposed to fermentation broth. (*c*) Optrode type sensor protected from fermentation broth, but requiring direct exposure of its biosensitizing membrane. (*d*) Biosensor protected by a microporous filter or dialysis membrane (flow lines for calibration can also be added). (*e*) Continuous-flow dilution probe allowing dilutions in the range 100- to 1000-fold. (*f*) Direct (semi) continuous sampling of fermentation broth. (*g*) Direct (semi) continuous sampling of fermentation broth and removal of particulate matter by filtration.

configured within an otherwise robust probe (figure 1*d*, *e*), allowing routine calibrations to be carried out on line and avoiding the need to sterilize the biosensors. Such membrane probes can be operated without flow (Enfors & Cleland 1983), when analytes in the fermentation broth equilibrate within the biosensor compartment, or, where the biosensitization layer is immobilized, they can also operate by continuous-flow dilution (Clarke *et al.* 1982, 1985). Continuous-flow dilution has the advantage of continuously bathing the sensors in appropriate buffer solutions and can allow the biosensor devices to be sited slightly downstream, to facilitate their maintenance and removal during *in situ* steam-sterilization procedures.

A number of approaches for improving selectivity and/or compensating for interferences have been considered. Procedures to sequester interfering substances are not generally wholly successful. In the case of chemical sensors, reference sensors responding to interferents

in a similar fashion to the working sensor have proved useful. Sensors have also been calibrated for major interferents and the resulting data matrices inverted to correct for interferences by continuously monitoring the interferents with separate sensors (Clarke *et al.* 1982). A related approach has been to use a large array of non-specific sensors which, together with appropriate data processing, can recognize particular chemical species (Cary *et al.* 1986).

Physical measurement techniques, especially optical, acoustic or dielectric, largely conform well to the operational constraints of process-control design. Most such measurements can be configured so that the sensor or transducer need not be in direct contact with the medium and can therefore be encased in probe bodies of industry-standard materials such as stainless steel, ceramics and thermoplastics. These techniques also have the advantage that the measurement is relatively free from drift and can often be an absolute measurement, rather than requiring some form of routine user-calibration. The operational life of such probes would also be expected to be high. Consequently, monitors based on direct use of physical principles are more likely to make greater impact, at least in the short term, than true biosensor devices.

However, compared with biosensor devices, the specificity of physical measurements can be problematical. Particularly in the case of physical measurements responsive to bulk, macroscopic culture characteristics and interferences from non-biological material (bubbles, precipitates, cell debris, medium particles and emulsions) can be significant. Some of these problems can be overcome by using digital signal processing and analysis techniques (e.g. time- or frequency-domain spectrum analysis), where the various interferences can be characterized as responding differently. Further, when the heterogeneity of cell populations is being examined by means of individual cell-analysis techniques, rather than by the aforementioned analysis of bulk macroscopic parameters, the rejection of signals from interfering materials in the measurement volume can be made more effective.

4. Development of suitable biosensor devices

A wide range of biosensor devices has been reported. Most such devices involve immobilizing a biosensitization layer close to the conventional transducer, which can then convert changes in analyte levels to an analogue electrical signal. Although devices can be based on electrochemical, optical, optoelectronic, piezoelectric or thermistor transducers, most have this common configuration and overall principle of operation.

Solid-state devices can be constructed by immobilizing (chemically and/or by encapsulation in polymers) the biosensitization layer on the sensing surface of the base transducer. Although this approach is more elegant, in some regards, simple entrapment of the biosensitizing solution (e.g. enzyme) behind a dialysis membrane can be more applicable in practice. The fermentation probe can be made robust and steam-sterilizable, without removing the base transducer (e.g. pH or amperometric electrode). The enzyme solution can be introduced into the probe straightforwardly and can be easily replaced (Enfors & Nilsson 1979).

Whether biosensor devices are solid-state or liquid-filled, they possess similar problems, mainly the denaturation or loss of their biosensitization layers and interferences both at the levels of the biosensitization and base transducer. These problems are very difficult to overcome within the confines of the above overall configuration and principle of operation. Therefore, rather than devoting considerable effort to minimizing these problems, it is perhaps more appropriate to develop better principles of operation.

Several groups are now developing biosensors in which the coupling between the biosensi-

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tization element and the base transducer is more direct. Amperometric biosensors are the most developed in this regard. Ferrocene has been used to mediate electron transfer in glucose-oxidase-modified carbon electrodes (Turner 1983; Davis 1985). This type of biosensor has been further developed to be reliable enough for fermentation monitoring (Brooks *et al.* 1987). Direct electron transfer between enzymes or redox proteins and electrodes can be promoted by agents that hold proteins and their active centres close to electrode surfaces (Hill 1983). A further, more recent approach is to couple electron transfer directly to organic conductor electrodes (Albery *et al.* 1985). Although these approaches achieve more direct mechanistic coupling between biosensitization and base transducer, thereby improving the performance of the biosensor, the degree to which close spatial coupling is achieved can vary; biosensitization and electrode still tend to be distinct layers. Large areas of the base transducer also continue to be exposed to medium; interference at the 'electrode' level can therefore still be a problem. Attempts are being made to further these developments by coupling organic conductors and enzymes at the molecular level (Faulkener 1984). This would be expected to minimize the interference further, as the base transducer would approach a 'molecular electrode'. One approach is to encapsulate enzyme molecules within polymer chains that have redox centres. The extension of this would be to interconnect enzyme molecules and the polymer chains with mediators or organic conductor molecules bound close to the active centre of the enzyme. Immobilization methods that encapsulate the enzyme within polymers tend to be more protective, minimizing denaturation and loss of the biosensitization elements. However, present encapsulation techniques tend to create lacunae containing many enzyme molecules. Encapsulation of individual enzyme molecules at short molecular distances to redox polymer chains, while maintaining sufficient transport to the encapsulation site, continues to be a problem.

Biological electron-transport systems achieve molecular coupling effectively in bilayer membranes. The coating of devices with molecular films of amphiphiles of similar nature to membrane phospholipids is now well established on the basis of Langmuir film technology. Although a number of integral and peripheral membrane enzyme systems can now be reconstituted in artificial membranes, their co-incorporation in Langmuir film coatings continues to be problematical. A further drawback is that most biosensitizing agents presently used are hydrophilic and cannot be incorporated into such layers without substantial chemical or biochemical modification.

Potentiometric biosensors are far less developed; more direct coupling principles have not been convincingly demonstrated. However, biological membrane systems again use such principles. Membrane transport proteins are analogous to those phase-transfer catalysts responsible for the principle of liquid membrane ion-selective electrodes. Although some have attempted to incorporate hydrophilic enzymes into polymer supported liquid membranes by using plasticized poly(vinyl chloride) (Alexander & Joseph 1981; Alhitti *et al.* 1984) the underlying mechanisms are not those of permselective ionic semiconduction. Instead, close spatial coupling of redox reactions and changes in ion levels are responsible. However, an interesting glucose sensor has been prepared by incorporating a barium–glucose salt into an amphiphile plasticized poly(vinyl chloride) membrane (Wilkins & Wilkins 1983). Micelle techniques perhaps represent an easier way of reconstructing the processes occurring at biological cell membranes by means of hydrophilic (or amphiphilic) biosensitization elements.

Fewer biosensor devices for proteins have been demonstrated. The binding of proteins to titanium oxide semiconductor electrodes, modified with affinity dyes, produces small potentio-

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metric signals (Lowe 1984); similarly small signals result from protein binding to field-effect transistors (Janata 1975). A combination of charge leakage around the interacting molecules and the distance of the interaction from the charge-sensing surface are likely to be the cause of these small signals. Further, antibody–antigen interactions are not accompanied by large conformational changes, which, in other interacting systems, could serve to transmit binding to the charge-sensing surface. An alternative approach, producing larger signals, has been to modify liquid–liquid interfaces with affinity ligands, by attaching hydrophobic 'tails' to the affinity ligands (Schiffrin *et al.* 1986). Colorimetric changes have been used to detect proteins (Goldfinch & Lowe 1980), which could be applied in fibre-optic or optoelectronic configurations. However, a more widely applicable technique is the use of plasmon-resonance affinity sensors (Place *et al.* 1985). Microgravimetric affinity biosensors based on piezoelectric crystals also appear to be promising (Roederer & Bastiaans 1983).

More direct mechanistic and spatial coupling principles are being developed, which would be expected to improve the performance of biosensor devices. Although most attention has been devoted to solid–liquid interfaces, more recent interest in reproducing processes occurring at biological cell membranes, by means of liquid–liquid interfaces and membranes, also appears promising, particularly as these are potentially much easier to manipulate.

5. Physical monitoring techniques

In discussing process control there has been a tendency to concentrate on fermentation monitoring. Monitoring and analysis of particles and organisms in gas and fluids throughout the process plant is also important (i.e. in medium-preparation tanks and lines, culture storage and processing, cell harvesting, cell breakage and debris harvesting).

(*a*) *Acoustic techniques*

Acoustic propagation and scattering techniques have long been used for monitoring fluid systems. Although biological applications are apparent, comparatively few developments have been made. Ultrasonic absorption spectroscopy has been used to analyse the ionization and conformation of proteins (Cho *et al.* 1985). Yeast culture growth has been monitored by means of a pair of piezoelectric transducers, which appeared to respond more to the compressibility of the suspension, than to the sound velocity or suspension density (Ishimori *et al.* 1981). A number of other groups are developing multicomponent analyses of macroscopic scattering and propagation parameters to extract useful information (e.g. biomass concentration, differentiating cells from bubbles and other particles, cell size and shape, medium viscosity and density) (Clarke *et al.* 1987). Some acoustic parameters can be monitored non-invasively and probes can remain relatively free from fouling because the probe can be made to vibrate (Blake-Coleman & Clarke 1985). Ultrasound beams can also be focused (Wickramansinghe 1984), thus potentially providing a means of population analysis. Acoustic-resonance densitometry techniques provide a high-resolution means of monitoring density in the absence of viscosity components. When medium density and suspension density are measured simultaneously, biomass concentration can be observed over a very wide range, suitable for monitoring both fermentation and processing of cell slurries (Blake-Coleman *et al.* 1986).

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(*b*) *Dielectric spectroscopy*

Monitoring of dielectric and conductimetric properties over low-frequency to near-radio frequency ranges (figure 1) provide dielectric dispersion data which can be computed to give the biomass volume fraction (Kell 1986). These frequency-dependent changes have two main regions of interest, which report on the dielectric properties of the cell envelope and cell membrane. These spectra could well be of value in analysing the condition of cell suspensions. However, a combination of fouling and interference at naked metal electrodes, together with the lowering of measurement resolution by high background medium conductivities, has made their application difficult. Nevertheless, monitoring of such properties non-invasively and at high resolution in relatively conductive media can be achieved (Blake-Coleman *et al.* 1987).

(*c*) *Laser light scattering and related techniques*

Although well-established optical techniques (e.g. spectrophotometry and turbidometry) are used widely for biological laboratory work, only spectrofluorimetric monitoring of cellular cofactors (Beyeler *et al.* 1981) and infrared absorption techniques (Mullard 1984) have been considered as fermentation monitors.

More sophisticated laser techniques can perform some population distribution analyses in bulk samples (Carr *et al.* 1986*a*). Photon correlation spectroscopy (PCS) is capable of limited size-distribution analyses for organisms smaller than 3–10 μm, for example, allowing culture contamination to be identified in the range of one part in 250 to one part in 1000, depending on the relative size of the organisms. Fraunhöfer light-scattering systems also operate on bulk samples, achieving similar performance for organisms of size greater than 1–5 μm. Although of lower absolute size accuracy than both of these techniques, much higher-resolution analysis of population distributions could be achieved by counting and analysing light scattered from individual cells passing through a laser beam of diameter similar to the size of the cells.

Circular-intensity differential scattering (CIDS) analyses multiple vectors of scattered light, such as polarization and angular scatter, and can monitor the quantity and state of coiling of chiral molecules such as DNA (Saltzmann & Gregg 1984). Although effective in DNA solutions, the analysis of DNA inside virus particles was of lower resolution because of the light scattered by the protein coat of the virus. Application to whole cells would be expected to be more difficult, because most of the incident light is scattered by the refractive index boundary at the cell envelope.

The erosion of the quality of light-scattering data by the natural polydispersity of microbial cultures (e.g. non-spherical cells, cell elongation and chaining) can be overcome to some extent by using multiple angle measurements. Ultrasound fields can be used to concentrate cells into bands (Vienken *et al* 1985); under similar conditions, cells also polarize along their major axis (W. T. Coakley, personal communication). The use of multiple angle measurements would then become a more powerful technique. In analogous fashion to the enhancement of laser-based flow cytometric techniques, by treating cells with specific fluorescent reagents, similar techniques could be used to enhance some of the above bulk-sample laser light-scattering techniques, potentially providing rapid diagnostic analyses on bulk samples.

PCS is also capable of analysing the size and polydispersity of protein and other biomacromolecule solutions (see, for example, Brown 1986; Carr *et al.* 1987). Laser Doppler velocimetry is a similar technique capable of monitoring the motion of solutions and suspensions, providing

a flow monitor that is widely applicable in process plants and a means of rapidly monitoring electrophoretic mobility. These techniques appear to have an important role in monitoring concentrations, fluid flow rates, molecular size, polydispersity and charge in protein purification systems and are a significant improvement on conventional UV monitors.

A major drawback of sophisticated laser light scattering techniques has been the high capital cost of equipment and its availability only in research configurations. Fibre-optic sensors have been developed to allow remote, multisite analyses, rendering the systems more cost-effective and easier to apply. Avalanche photodiodes can now replace photon-counting photomultipliers; in a similar way, low-cost laser diodes are being considered as replacements for lasers (Brown 1986).

6. Conclusions

Developments in process plant monitoring techniques are particularly apparent for cell growth and population analysis, primarily based on physical measurement techniques. The combination of acoustic, dielectric and laser techniques appears to have the potential to monitor cell concentration and cell condition, and to analyse the nature of populations. Although applications for a number of existing biosensor and chemical sensor devices can be achieved by using membrane-sampling and flow-probe configurations, the further development of more directly coupled and reliable biosensor devices is expected to have a greater impact. Although laser techniques offer a range of monitors for downstream processing, more extensive development of enzyme and protein biosensors is required.

The comparatively poor stability of most enzymes is a major drawback to the application of biosensor devices in continuous monitoring. Surprisingly little attention has been devoted to chemically and thermally more stable enzymes isolated from thermophilic organisms. Protein engineering is providing the ability to alter the kinetics and properties of enzymes by site-directed mutagenesis and to develop potentially useful protein fusions (e.g. biochemical-assay amplifying enzyme fused to Protein A).

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Discussion

I. Bergman (*Health and Safety Executive, Broad Line, Sheffield, U.K.*). Dr Clarke raised two points of interest in the field of application of sensors: sampling and steam sterilization. We recently developed an amperometric sensor for sulphur dioxide and nitrogen monoxide to be used directly in flue gases at 280 °C. Such a sensor could be used for oxygen and for many other gases after repeated steam sterilization. Just as with fermentors, sampling lines for

monitoring are unpopular in the flue-gas field also. Could I ask Dr Clarke to comment on the possibilities of alternative methods of sterilization, and on whether any methods can be used for sensors containing biological materials?

D. J. Clarke. Although mass spectrometry is now used to monitor a range of volatile species and gases, both in the gas phase directly and as dissolved species (by means of gas-permeable membranes), there still appears to be room for specific gas-sensor devices. Where these operate at high temperatures, as well as surviving heat sterilization, they could perhaps operate more effectively in the presence of the high levels of water vapour in fermentation exhausts. However, the majority of fermentation sensor requirements are in the fluid phase. Ideally, these sensors should be applied directly in the broth, raising not only the problem of sterilization but of sensor reliability in such complex media (compared with the less complex gas phase). Flow-dilution or equilibrium diffusion-membrane probes avoid the need to sterilize chemical sensors and biosensors, allow routine recalibration on line and provide a more protected environment for the sensor devices, while minimizing the use of sampling lines. Where these sensors are reliable enough to be applied directly, sterilization then becomes imperative. Steam sterilization is the only practice widely accepted in the fermentation industry. Some solid-state chemical sensors can survive autoclaving to some extent, whereas only biosensors containing thermostable enzymes with low water content could perhaps survive less aggressive heat sterilization. Chemical sterilization can be less effective with complex surfaces. Further, many reagents used also tend to be deleterious to biosensors and can also affect electrochemical responses, pointing to the potential alternative use of irradiation to provide aseptically packaged sensors. It appears likely that the fermentation industry would also require effective and simple means of aseptically applying sterile sensors before such an approach would be acceptable.

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