death. Listing similarities alone does not help to understand evolutionary differences.

One of the ways to encourage this kind of thinking in biochemistry is to promote communication across the specialized fields of research. Active development of scientific communication is not encouraged by science education. To counter this, the organizing committee of this congress had arranged for a session on new concepts in teaching biochemistry. Of all the speakers, F. Vella (University of Saskatchewan, Saskatoon) tried to shake those scientists who believe that the act of doing science is something beyond the realm of mere mortals: on the contrary, scientists have an obligation to make science understandable to others so that they can learn from it, judge it and contribute towards its development.

The modern era of biotechnology is a proof that biochemistry is not an isolated intellectual activity. Science has a direct bearing on society which in turn has the final say over science. Combining scientific knowledge and social awareness is the only humane way of making biotechnology a tool for improving, at least, the material conditions of mankind.

Acknowledgement

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Reference

1 Rattan, S. I. S. and Clark, B. F. C. (1988) *Trends Biotechnol.* 6, 58–62

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TOOLS OF THE TRADE

Liquid emulsion membranes for concentrating biochemicals

Liquid emulsion membranes (LEMs) consist of an emulsion of droplets in an oil-immiscible (aqueous) continuous phase. The droplets themselves are a water-in-oil phase. Thus the oil provides a liquid membrane between two aqueous phases across which solutes can be transported, thereby achieving separation. By adjusting the volume ratio of the external and internal aqueous phases, LEMs can be used to concentrate and separate solutes.

Phenylalanine was used as a model system to study the effects of process parameters on LEMs. It was concluded that LEMs could be used effectively to separate and concentrate biochemicals even from fermentation broths which have undergone minimal or no pretreatment.

Thein, M. P., Hatton, T. A. and Wang, D. I. C. (1988) *Biotech. Bioeng.* 32, 604–615

Fluorescence sensing in fermentation with fibre optics

In the usual approach to fermention monitoring by fluorescence, fluorescence indicators have been immobilized on fibre-optic probes behind semipermeable membranes. This has made their performance similar to other types of electrodes.

An alternative approach is to dissolve an array of fluorescence indicators in a fermentation broth and to use a single fibre-optic probe placed directly in the fermentation system to detect a range of different parameters. The following fluorophores were used to facilitate the measurements: pyrene butyric acid (dissolved oxygen); HPTS (pH); D-tyrosine (phosphate); D-tryptophan (temperature); 4-methylumbeliferone (broth absorbance). In addition, the cell density could be estimated using a right-angle probe to detect scatter spectra. These fluorophores are autoclavable and appear to have no effect on cell growth in microbial or animal cell cultures.

Junker, B. H., Wang, D. I. C. and Hatton, T. A. (1988) *Biotech. Bioeng.* 32, 55–63; the authors are at the Department of Chemical Engineering, MIT.

Alginate–silica composite gels

Calcium alginate gel for entrapping various types of cell can be prepared easily under mild conditions. One of its drawbacks, however, is its deformability which limits its use in high volumetric scale reactors. Silica gel on the other hand is much harder but cannot be used to entrap cells. A composite of silica and alginate, however, combines properties of both gels.

To a colloidal silica solution adjusted to pH 7–8 was added 3% alginate solution and distilled water to obtain a final alginate concentration of 1.5% and a final SiO_2 concentration of 5–20%. This solution was dropped into 5% $CaCl_2$ and left to stand for 5 h to form beads.

The beads formed were intense and hard, making them useful for plug flow operation. The pores of the composite gels were smaller than those of comparable alginate gels. The composite gels have been used to immobilize yeast for ethanol production and to absorb enzymes.

Fukushima, Y., Okamura, K., Imai, K. and Motai, H. (1988) *Biotech. Bioeng.* 32, 584–594; the authors are at the Kikkoman Corporation and Fuji-Davison Chemical Ltd.

Electronic measurement of cellular biomass

The real-time estimation of microbial biomass in laboratory and industrial fermentations is still a problem. A recent article[1] describes how the dielectric permittivity (i.e. the electrical capacitance) of cell suspensions at low radio frequencies is a linear function of the concentration of the suspended phase (i.e. the biomass); at the same resolution permittivity is, for practical purposes, independent of the concentration of non-cellular particulate matter and gas bubbles. This is because, of the components likely to be found in fermentors,

only biological cells are surrounded by lipophilic membranes of molecular thickness which possess a high electrical capacitance.

The accurate registration of electrical capacitance in ionic solutions requires only a four-terminal metallic electrode arrangement and an appropriate impedance-measuring instrument. The principle of using capacitance for measuring biomass therefore opens up the possibility of producing a non-invasive, real-time biomass probe with no moving parts. Because the electrodes are metallic, they may be cleaned electrolytically *in situ* to prevent biofilm formation. An instrument based on these principles, the βUGMETER, has been developed specifically for use in the estimation of cellular biomass in fermentors, and is commercially available from Aber Instruments, Aberystwyth Science Park, Aberystwyth, Dyfed SY23 2AH, UK, tel. (+44) (0)970 615284.

Harris, C. M., Todd, R. W., Bungard, S. J. *et al.* (1987) *Enzyme Microb. Technol.* 9, 181–186

Contributed by Douglas B. Kell, Department of Biological Sciences, University College of Wales, Aberystwyth, Dyfed SY23 3DA, UK.

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LETTERS

More on fusing plant protoplasts

The recent *TIBTECH* review by Michael Jones of methods for fusing plant protoplasts[1] is somewhat dismissive of other methods of fusion. The methods that are described in much greater detail are a subset of electrofusion methods in which electric fields are used firstly to aggregate cells (through alignment) and then to permeabilize them, leading to fusion. Aggregation can, however, be induced other than with electric fields. Techniques involving aggregation by sedimentation[2] or by the use of a very low concentration of an agglutinating agent such as PEG[3] have been known for some time and are easy to use. They can lead to the generation of large numbers of viable hybrids[2,4–7].

I will describe briefly the simple procedure for fusing cells using agglutination agents. (1) Protoplasts are suspended in an electrofusion medium in which a small amount of an agglutinating agent (PEG 2.5% w/v) may be present. (2) A small volume of the suspension (0.2 ml routinely) is transferred to a Petri dish or a pulsing chamber and left to settle. (3) After 10 min, the electrodes are brought into contact with the bottom of the dish with the protoplasts between the electrodes. (4) Suitable electrical pulses are applied. (5) The electrodes are removed and the pulsed suspension is incubated for 30 min at room temperature. (6) Culture medium is then added. Several drops of cell preparation can be pulsed on the same dish and several dishes can be pulsed in series.

The method permits complete control of both the electrical factors (intensity, duration, delay) and, importantly, the direction of the pulses. One advantage of this technique is that there is no limitation in the choice of the buffer in which the pulsing is performed as long as the viability of the protoplasts is not affected. (Not all agglutinating agents are appropriate; for instance, spermine has some adverse effects on cells.)

As described by Jones[1], the Joule heating effect means that dielectrophoresis should be operated only under conditions of very low ionic strength. This is not the case with the 'agglutination' approach where the pulsing medium can be pH buffered and contain a high ionic content (25–50 mM NaCl giving a high yield of viable hybrids[4]). Any ionic species can be present although Ca^{2+} was shown to be deleterious for viability[3]. The osmotic pressure can therefore be optimized.

The agglutination methods have other positive aspects. Large protoplast volumes can be treated (0.2 ml in a couple of seconds on a routine basis). Electrofusion is under the control of the electrical parameters and the composition of the pulsing buffer. Control of the fusion by the pulse duration (with the use of square-wave pulses) was described for the agglutination procedures some time ago[3]. Multifusion can be eliminated by working under conditions of low protoplast concentration[3].

Creating the contact between protoplasts by agglutination has already