The Role of Ion-selective Electrodes in Improving Fermentation Yields

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The use of pH and oxygen electrodes has now become a standard practice in fermentation research and production. Less prevalent, however, almost to the point of nonexistence, is the use of electrodes sensitive to other species as elements in systems for the monitoring and control of fermentation processes. With the ready commercial availability of numerous ion-selective electrodes, much progress may be expected in improving fermentation yields through optimisation of cultural conditions by incorporating these sensors into biochemical reactors.

"It is becoming apparent, largely as a result of the application of automatic instrumental control of pH and chemostat culture, that call properties can change profoundly over a narrow pH range. However, the molecular basis of these effects is little understood[1] ".

Introduction

That the foregoing quotation is something of a truism does little to hide our relative ignorance of the processes of microbial physiology, yet provides one with the germ of the notion that, given study, the researcher can improve dramatically the yields of desired products in microbial fermentations by the application of simple control engineering principles to growing cultures. Indeed it is now inconceivable that one might carry out an industrial fermentation without due regard to, and provision for, the control of culture pH, and, with equal universality, this is accomplished by the use of a glass electrode, immersed in the culture, which can sense the pH and, should this change from the desired value, activate a valve or pump which will add a reagent to return the pH to the desired set point.

However, there are many other electrodes now commercially available, which sense the concentration of dissolved species other than the hydrated proton, and the underlying thesis of the present article is that the judicious use of these so-called ion-selective electrodes (ISEs), additional to the commonly-employed pH- (and, to a lesser extent, oxygen-) sensitive electrodes, can bring about enormous increases in the yields of desired processes by their incorporation as sensors in feedback control loops, with the attendant economic advantages.

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Nature *vs* nurture

There are two main ways in which the productivity of an industrial fermentation may be enhanced; (a) by mutation and selection for high-yielding strains, that is to say by genetic manipulation, and (b) by optimising the chemical and physical environment of the fermenter during the fermentation process. That the scope for these two approaches is large is evidenced by the fact that, over the last 35 years the titre of penicillin G in commercial fermentations has increased from 2 units/ml to 50,000 units/ml[2] and, faced with such a statistic one might be tempted to conclude that no significant further improvements in the efficiency of the penicillin fermentation might reasonably be expected. However, as has been pointed out elsewhere[2], such a conclusion would be greatly in error, and, in a typical run it is found that only 6% of the glucose added is used for penicillin production, the rest being used for biomass production (c. 70%) and maintenance energy[1] requirements (c. 25%). Thus[2] "the actual yield of penicillin from glucose is an order of magnitude from the theoretical value, and there is room for substantial improvement. Increased efficiency of glucose utilisation for penicillin will also markedly decrease the demand for oxygen. This is important not only to reduce the production cost, but also to increase the capacity of existing equipment for penicillin production, since it is often the oxygen transfer ability of a fermenter which is rate-limiting". Thus we may conclude that even in the most highly studied and competitive fermentations there is a great deal of room for optimisation of the yield of desired product. To what extent can this improvement be attained by the use of cultural control?

Leaving aside the possibility of engineering a reactor configuration appropriate to particular product specifications, as recently reviewed[3], and assuming throughout a relatively idealised continuous stirred tank reactor (CSTR), operating either in batch or continuous mode, it is the purpose of the present section to remind readers of the types of increase in yield which may be obtained by the optimisation of the nutritional (and physical) environment of the fermenter. Since the pioneering work[4] which showed that the enzyme complement of *Escherichia coli* altered dramatically depending on the ambient external pH, it is now universally accepted, as pointed out in the opening quotation, that microorganisms can change their properties, including the yield of desired fermentation product, markedly over a narrow pH range. A further early example is given in the comprehensive studies of the effect of pH on the yield of the lactic acid fermentation[5,6]. Whilst the relationship between internal and external pH remains largely uncertain, and is possibly different for aerobes and anaerobes[7], an appreciation of the possible energetic role of a transmembrance pH gradient[8] has brought about a measure of increase in our understanding of the nature of the microbe's control of intracellular pH. What is clear, however, is that a change in the external pH of a microbial suspension will affect intracellular and extracellular enzymes in a different fashion.

Just as the significant effect of pH on microbial physiology has become an accepted phenomenon (see ref. 9 for other examples), so too has the role of dissolved oxygen concentration in affecting microbial metabolism become appreciated (Reviews:[1,10-15]). Apart from the cost of providing more oxygen than is required for optimum product formation, alluded to above, the ambient oxygen tension can greatly affect the pathway of microbial metabolism. Thus by using oxygen-enriched air it was found[16] that the maximum yield of dihydroxyacetone from glycerol in a *Gluconobacter* fermentation was almost double that from an oxygen-limited culture. Conversely, by keeping the dissolved oxygen tension limiting it was observed[17] that the specific yield of the purple membrane protein bacteriorhodopsin, in cultures of *Halobacterium halobium,* could be enhanced by a factor of approximately 5. It may justifiably be concluded[1] that the control of oxygen and pH has contributed, and can contribute, to a marked improvement of the channelling of microbial metabolic activities in desired directions. Of chemical treatments, as opposed to factors such as temperature[18], reduced pressure[19] or "dialysis culture[20]", only the effect of redox potential[21] on microbial activity has received anything approach-

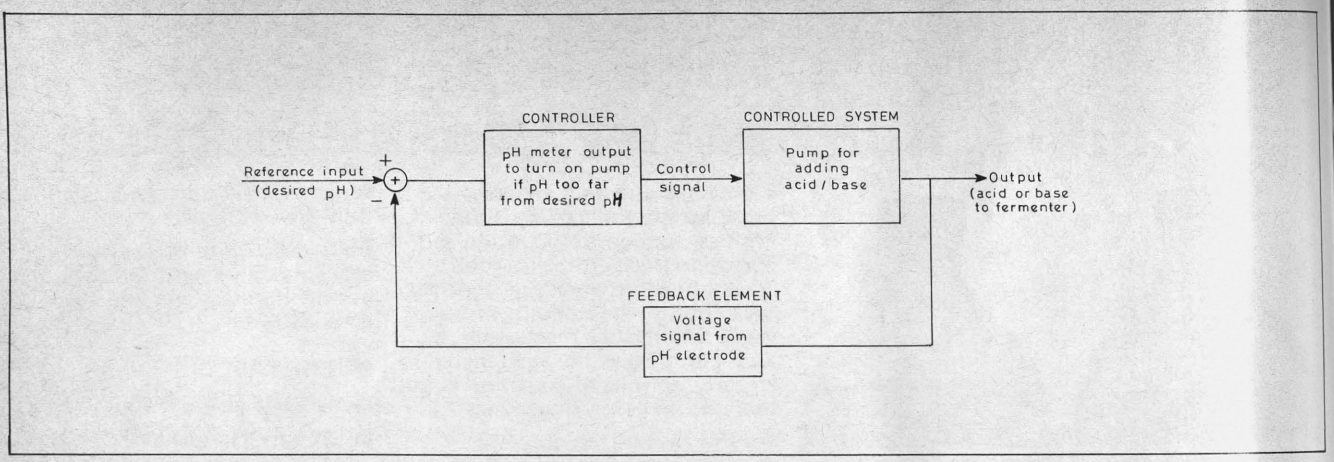

Figure 1. The principle of a negative feedback control loop, illustrated with reference to pH control.

ing a measure of attention. We would submit that, just as the control of pH, oxygen tension and redox potential may be used to optimise[22] both the physiological and economic aspects of a fermentation, so too by the use of other control systems can fermentations be similarly enhanced. In particular, the availability of numerous electrodes sensitive to species other than H^+ provides a significant opportunity for the fermentation scientist and microbial physiologist to achieve the optimisation of their fermentation systems by cultural techniques alone. Therefore, before considering the types of ion-selective electrode now available, it is appropriate to consider briefly the principles of the art of controlling the chemical environment of a fermenter.

Control theory

The underlying structure of a simple negative feedback control loop[23,24] is shown in Figure 1, both in general terms and as applied to the control of the pH[25] of a microbial culture in which a pH electrode is immersed. It is convenient to assume that the culture pH is only drifting in a single direction. As the pH reaches a preset value, the pH meter activates a pump which adds a certain amount of acid or base until the pH is again at an appropriate value. Delay loops are often introduced into this system so that sufficient time for mixing is allowed to elapse before the meter again 'listens' to the pH electrode and chooses whether or not to activate the pump again. This is the essence of pH control, and similar remarks may be made concerning the control of dissolved oxygen tension or redox potential; a sensor detects the ambient value of the parameter, compares it with a reference (desired) value, and then chooses whether or not to activate a system that will return the value of the parameter to the desired value.

It is clear, therefore, that in principle identical systems to that used for pH control may be used to attain the control of pH (specific ion concentration), save for the use of an ISE in place of the pH electrode. We therefore now turn to a discussion of some of the types of ISE which are now commercially available or have been described.

Ion-selective electrodes[26-32]

It has become conventional to classify ion-selective electrodes into the various types shown in Fig. 2. The key property of every ISE is that it possesses a membrane which exhibits *permselectivity* to a restricted number of species, ideally to only the determinand[26-33]. Only a few examples of each type of ISE are included in Figure 2, although many more species may be determined directly using the appropriate electrode. The mechanism of potential generation by ISEs will not be discussed in detail here (see 26 to 33).

In each case it is necessary to have a reference electrode against which the potential of the ISE may be determined. For most applications the calomel or Ag/AgC1 electrodes exhibit a potential independent of the determinand concentration and are therefore suitable. A typical electrochemical 'cell' containing an ISE is shown in Figure 3. When two phases containing electrically charged particles come into contact an electrical potential difference develops at their interface. The potential across the permselective membrane (Fig. 3) is given by the familiar Nernst equation:

$$E = \text{constant} + \frac{RT}{zF} \ln \frac{c_i(1)}{c_i(2)} \quad \ldots \quad (1)$$

where R, T and F have their usual thermodynamic meaning, z is the charge of the determinand ion and the concentrations (strictly, activities) of ions in phase 1 and 2 are respectively $c_i(1)$ and $c_i(2)$. It is clear that the potential exhibited by the ISE relative to its reference electrode will change if $c_i(1)$ is held constant and the potential of the reference electrode remains unchanged. This is the principle of the use of ISEs. The factor RT/F has a value of approximately 60 mV at 30°C. Thus the potential of an ISE changes linearly with the *logarithm* (Fig. 4) of the determinand concentration. This is indeed one of the many advantages of the use of ISEs: that a very large concentration range may readily be encompassed, typically 6 orders of magnitude. Some of their other merits are listed in Table 1[34], which alone may be said to justify the belief, elaborated here, that ISEs can constitute a control element of great utility in fermentation processes.

Pausing amidst the present eulogic flight, however, it is important to consider possible problems associated with the use of ISEs, both in general and in the special case of microbial fermentations. In particular the effect of interfering substances, drift and recalibration will be considered, all of which relate to the precision, and, more important, to the accuracy of the methodology. The author holds that it is totally inappropriate to attempt to calibrate an electrode in absolute terms, but that calibration must be carried out with standard solutions prior to the start of the fermentation. Figure 4 shows a typical potential/concentration response curve of an ISE both in pure solutions of the primary determined and in solution containing, additionally, increasing concentrations of

1. **Solid State**
   (a) Glass (H^+, K^+, Na^+, NH_4^+, etc)
   (b) Other e.g. Ag/AgX for Cl^-, Br^-, I^-, Ag^+, $S^=$
   LaF_3 for F^-
   MS_2 for Pb^{++}, Cu^{++}

2. **Liquid Membrane**
   (a) With dissolved Ion-Exchanger
   e.g. Ca^{++}, NO_3^-
   (b) With neutral carriers,
   e.g. Valinomycin for K^+

3. **Gas-Sensing**
   CO_2, SO_2, NH_3, NO_X

4. **Enzyme Electrodes**
   e.g. glucose oxidase for glucose
   penicillinase for penicillin
   urease for urea

Figure 2. Various types of ion-selective electrode.

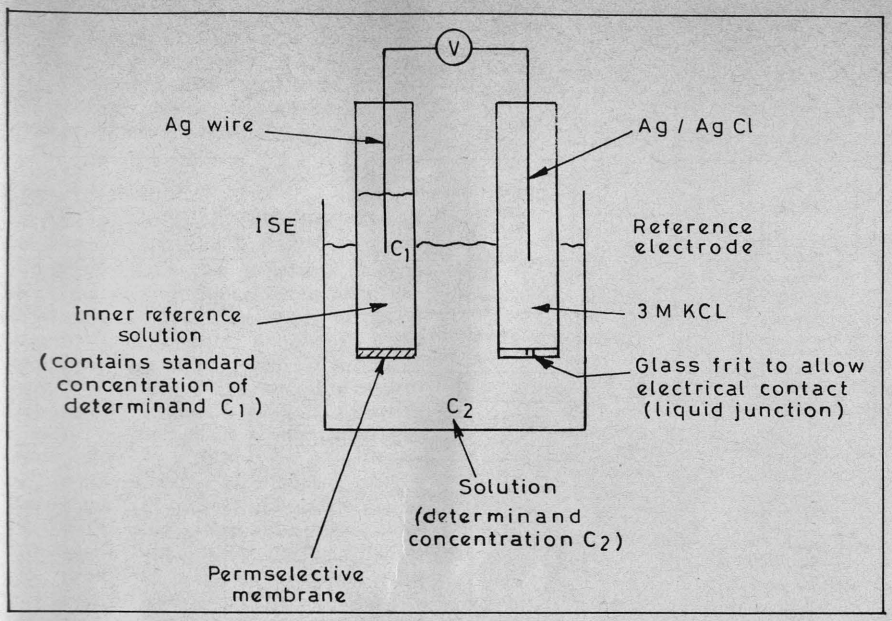

Figure 3. The principle of operation of an ion-selective electrode.

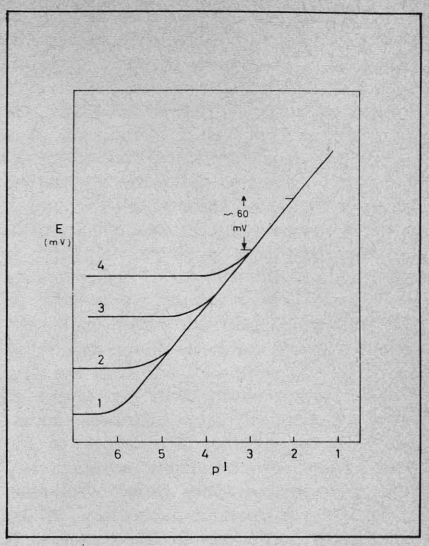

Figure 4. Typical response curves (potential vs concentration) for an ISE in the presence of increasing concentrations (curves 1 to 4) of an interfering ion.

an interfering ion. For the latter type of solution the Nernst equation is no longer valid, and the Nicolski equation (of which the Nernst equation is a special case) must be used. The Nicolski equation is as follows:

$$E = E_o + \frac{RT}{z_i F} \ln \left[a_i + \sum_1^n k_{ij}^{pot} (a_j)^{Z_i/Z_j} \right] \quad ..(2)$$

where the factor k_{ij}^{pot} is the so-called selectivity coefficient for the electrode between the primary determined i and any interfering substance j. The values of k_{ij}^{pot} can be found in manufacturers's literature, although it is recommended that for accurate work, particularly in complex media, that they be determined by the experimenter. It should be noted that some workers express the selectivity coefficient as k_{ji}^{pot}, so that the reciprocal of this selectivity coefficient must be used in equation (2). As a rule of thumb k_{ij}^{pot} is smaller the more selective an electrode is for i compared with j.

In general, it is appropriate to adopt something of a pragmatic approach to the use of an ISE in a new fermentation process, and first study the potential/concentration curves for the medium used in the absence of microbial activity. It will usually be the case that the concentration of the major interferents will change no more than that of the primary determinands as the fermentation proceeds. Similarly, drifting of the electrode response, insignificant in the short term but relatively extensive over periods used in continuous fermentations, can be noted in the absence of microbial activity. It may also be noted that the use of antifoam agents is not to be recommended when liquid membrane-type electrodes are being used, as these types of reagent, possessing weak detergent activity, will tend to extract the electroactive ion-exchanger from the electrode membrane. Little information has been published on autoclavability of ISEs, but manufacturers will readily offer guidelines. The effect of ionic particulate matter (such as microbial cells) in a solution is known to increase the noise of an ISE reading, and may also affect the liquid junction potential (Fig. 3)[28,29].

Table 1. Advantageous features of ion-selective electrodes for fermentation control.

1. Continuous, real-time assay.
2. Sensitive (usually to $<10^{-6}$ molar).
3. Electrodes biologically inert.
4. Non-destructive assay: no added reagent.
5. Many electrodes, using same equipment.
6. Selectivity; good to poor, depending on need.
7. Relatively rapid responding.
8. Good lifetime
9. Low cost.
10. Respond to thermodynamically important activities rather than concentrations.
11. Can be used in optically opaque and turbid suspensions.

Recalibration

Undoubtedly one of the least-studied areas in the field of ISEs is the question of how to recalibrate an ISE during a continuous fermentation under sterile conditions. One possibility, of course, is to take aseptically a sample of fermenter fluid and measure the concentration of the ion outside the fermenter, and adjust the 'apparent' pI to the 'correct' value. Whilst this approach is acceptable in principle, and advisable during early development of a new fermentation, a simple alternative is possible. This method, which requires only standard additions of substances and continuing recording of the electrode potential, has been outlined in principle in the case of an oxygen electrode[35]. Since its application to other electrodes is somewhat different and has not been described before, it is presented here in some detail. Imagine a continuously stirred tank reactor in which a fermentation is being carried out continuously, and the concentration of a particular ion is being controlled. The balance equation for the ion is given by:

$$N = k_1 (C - C^*) \quad ... \quad (3)$$

where N is the rate of usage of the ion by the microorganisms (mmol $hr^{-1}.1^{-1}$), C^* is the set-point concentration of the ion and C is the concentration midway between the set-point concentration and that obtaining immediately after the addition of ion via the control system. Thus C may be regarded as the 'average' concentration of the ion in the fermenter in a non-ideal case. N is obtained from the concentration of ion in the adjusting reagent and the amount added per unit time. k_1 is therefore derived from equation 3. Now the pump is actuated manually for a short time such that the actual concentration in the fermenter increases significantly above the set-point, and the time taken for the concentration to fall again to the set-point is noted. If this time is compared with that obtained during an earlier calibration, the relative values for the slope of the electrode in the Nernst (or Nicolski) equation are given (if N is constant) by the reciprocal ratio of the times. Thus, millivolt readings may be converted to 'real' concentration readings, by appropriate change of the meter settings. This method assumes, incidentally, that E_0 is unchanged. Whether this assumption is justified may easily be established by considering the shape of the trace for ambient pI, where changes in E_0 or the electrode slope may be picked up as changes in the rate of activation of the pump, extent of excursion of the trace assuming a standard amount of reagent is added each time, and changes in the sope of the apparent 'uptake' of reagent as the concentration drops again to attain the value at which the pump is reactivated.

Instrumentation

Allusion has only briefly been made to the type of instrumation necessary with ion-selective electrodes, largely because for simple cases a 'pirated' pH meter of reasonably high impedance, or a pH-stat apparatus, will serve adequately. Most commercial fermenters are equipped with this type of apparatus, and it is unnecessary to discuss its operation in any greater detail here. It is, however, germane to draw attention to what

the present author considers a key aspect of the use of multi-ion-control in fermentations. It is customary to use a different pH-meter and reference electrode for each voltage signal, be it from an O_2, redox, pH electrode or temperature probe. This is a completely uneconomic approach to instrumentation compared with the alternative, which is to use a voltmeter with a number of input channels which are rapidly scanned. In this way only a single voltmeter is required, and this can be of higher quality per unit of capital invested in instrumentation control. Additionally, the use of a digital voltmeter allows easy interfacing with a computer, and it is the view of the present author that the advent of relatively cheap microprocessor-based apparatus will be of great significance to the development of completely automatically controlled fermentations both in full-scale plant and the research laboratory. Whilst the use of computers in fermentations is by no means new[36–40], there have been few applications including the use of ISEs as sensors. The addition of these to the armoury of the researcher wishing to optimise his fermentations is long overdue. A lucid resume was recently given in this journal[41] of the philosophy and use for fermenter optimisation of computers. A system based on a scanning digital voltmeter under microcomputer control, as used in this laboratory, is diagrammed in Figure 5. At present only analysis rather than control is carried out, but the system allows real-time calibration of all electrodes, the obtaining of selectivity coefficients from calibration curves and inter-electrode correction of readings. However, the question of the necessity of control, rather than mere analysis, of fermentation parameters during a full-scale previously optimised, fermentation is an interesting one, and is one to which attention is now turn.

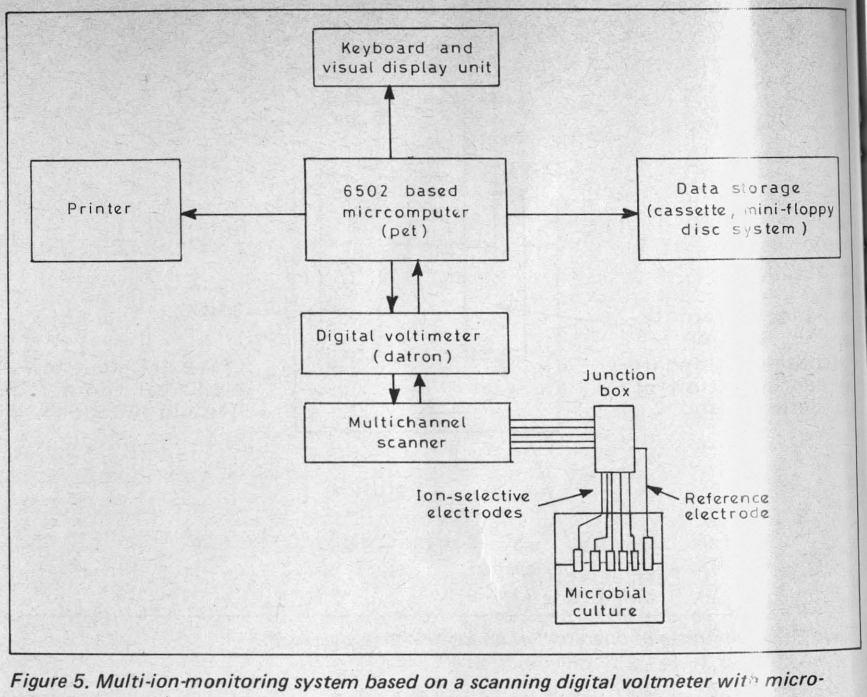

Figure 5. Multi-ion-monitoring system based on a scanning digital voltmeter with microcomputer control.

What is a true chemostat?

As every student of microbiology knows, a chemostat is a type of continuous culture system in which the composition of the inflowing medium is so arranged that the microbial activity inside the CSTR so reduces the concentration of a necessary substrate that growth is thereby limited. In the steady state, then, a low and limiting concentration of this substance is found within the fermenter, and thus, in principle, in the effluent stream[1,42,43]. For this reason, the term chemostat is used, for at a given dilution rate the concentration of a chemical in the fermenter (chemo) is held constant (stat). However, this chemical concentration, as sensed by an individual microorganism in a fermenter, is held low because of the activity of the other microorganisms in the fermenter, and *vice versa*. This may therefore be referred to as *intrinsic chemostat operation*. The alternative method, *extrinsic chemostat operation* may be realised by a system in which the level of a desired nutrient in the incoming medium is essentially zero, and is adjusted *extrinsically* to the set-point level via a control system based on an ion-selective electrode of the type described in Figure 1. This type of system is much easier to operate during the research phase of the optimisation of a fermentation since only a single minimal medium need be made up for the influent substrate, changes in the concentration of desired constituents being easily adjusted by changing the set-points of the pI on meter readings. This is in contrast to the intrinsic mode, where each change in fermentation medium constituent concentration requires a new recipe for the influent medium, and, moreover, one which bears no necessary relationship to that existing within the fermenter in the steady state. Once the final medium constitution *in the fermenter* has been optimised, it is then possible to adjust the influent medium to attain similar conditions in the intrinsic mode for use in the final, full-scale fermentation, if this mode be regarded as preferable.

Has extrinsic control of fermentations with ISEs improved yields previously?

The observant reader will point out that the type of extrinsic control system elaborated bove will result in significant oscillations of nutrient concentration within the fermenter, their extent depending on the delay times in the control loop, the rate of nutrient usage by the microorganisms and the concentration of nutrient added when the set-point is reached. Whilst this regime can wreak havoc on the interpretation of physiological studies in the chemostat[44], there is evidence[3] that this type of semi-continuous culture can have significant enhancing effects on the microbial production of secondary metabolites, possibly as a result of what have been called 'slip' reactions[9].

In any event, the ability of the experimenter to control the concentration of a particular metabolite by appropriate feedback signals, either regarding further nutrient additions or changing the flow rates of influent medium does offer the possibility of significantly enhancing the productivity of the economics of fermentation and waste treatment systems. Some examples follow which illustrate this view. Carbon dioxide is a well-known regulator of microbial growth and activity[11]. Whilst published studies of the effects of dissolved CO_2 concentration on fermentation processes have been few in number, attention may be drawn to the work[45] which used a CO_2 electrode to control the dissolved CO_2 concentration during an inosine fermentation, and found that inosine production was severely inhibited above 0.05 atm. A particularly obvious application of the CO_2 electrode is in the controlled growth of autotophic organisms, of which photosynthetic organisms for use in biomass production[46] or dinitrogen fixation to ammonia provide examples of current interest[47]. Similarly, of species which may be sensed using 'gas' electrodes, the regulation of fermenter ammonium concentration would seem to offer important economic savings, both from the point of view of the cost of nitrogen sources and from the fact that nitrogen-limited cultures tend to exhibit maximal secondary metabolite production[9]. Regarding other types of electrode, the elegant use of a sulphide electrode to monitor the methanogenic fermentation may be noted[48], and a very early innovative use of a cyanide-sensitive electrode to control the flow rate in a continuous microbial system for treating cyanide-containing effluent[49]. The uptake of both nitrate[50] and tetrathionate[51] by respiring bacterial cultures has been observed using ion-selective electrodes. Electrodes sensitive to detergents (e.g.[52]) are also easy to construct in the laboratory. The author knows of no studies which have set out specifically to investigate the role in affecting fermentation yields of simple cations such as K^+, Na^+, Ca^{++} and Mg^{++}, although electrodes selective for each of these species are commercially available. The utility of ISEs in continuously following microbially catalysed processes for the extraction of metals from low-grade ores[53–55] is sufficiently obvious to need no particular stressing.

It is regrettable, in view of the subtle effects of inorganic phosphate on microbial secondary metabolism[56], that no

electrode is available for direct estimation of this ion. It would be inappropriate to conclude this review without pointing out explicitly the potential utility of enzyme electrodes in monitoring continuously the progress of fermentation processes.

Enzyme electrodes

As pointed out above, the selectivity of an ion-selective electrode is governed by the permselectivity of the membrane dividing two aqueous solutions containing the determinand. The possibility of using enzymes to enhance enormously this selectivity has been recognised for some time[57]. The *modus operandi* of a penicillin-sensitive electrode is shown in Figure 6; a layer of penicillinase covers a pH electrode, either in a polyacrylamide gel layer by enclosure within a cellophane dialysis membrane[58] or by adsorption onto a glass frit[59]. Penicillin molecules diffusing into the region occupied by the penicillinase are hydrolysed, yielding penicilloic acid; the local pH changes and the pH electrode monitors this change. The electrode described previously[59] showed Nernstian responses to a range of penicillins with a linear relationship in the range 10^{-5} to 3×10^{-3} *M*; it was claimed that the electrodes functioned satisfactorily for six weeks without deterioration of their behaviour, and were suitable for use in fermentation broths.

Electrodes have been described for urea, sugars, amino acids and more arcane species such as amygdalin[60,61]. The variety of possible enzyme electrodes is limited only by the ingenuity of the experimenter. The use of polarised electrodes, working in the manner of a fuel cell, to continuously monitor methanol during Single Cell Protein fermentations may be pointed out[62]. Like the dissolved oxygen electrode, this is, of course, a polarographic electrode, where current is measured, in contrast with the other types of ISE considered here which are potentiometric, in which a voltage is recorded.

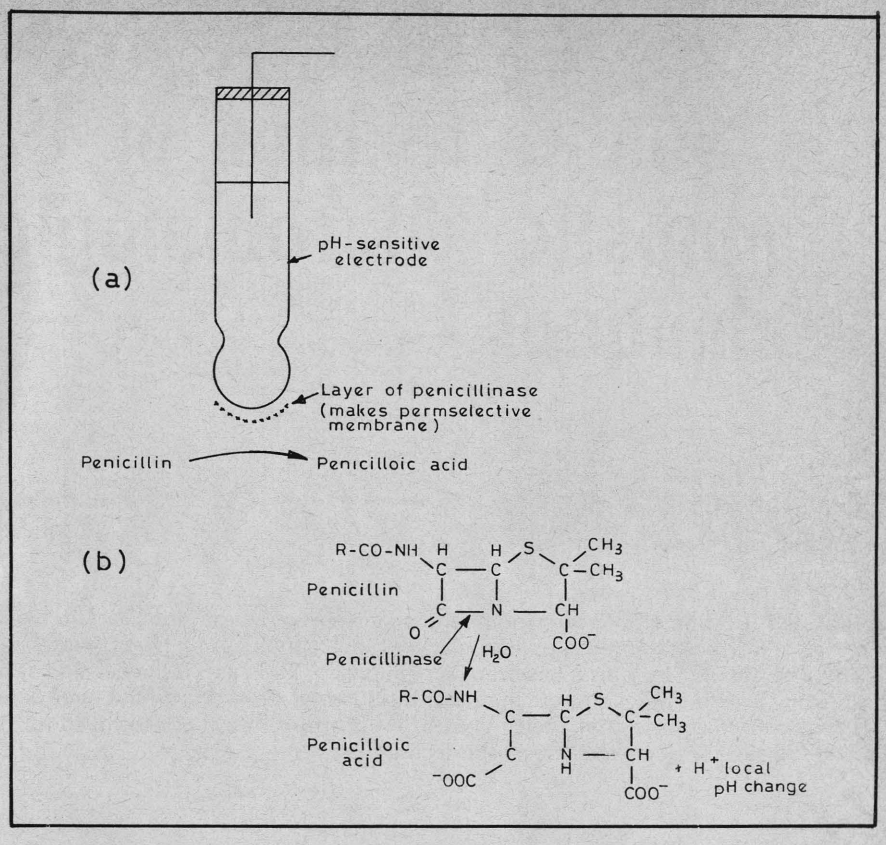

Figure 6. The principle of an enzyme electrode, illustrated by a penicillin electrode. (a) diagrammatic representation, (b) reactions occurring leading to electrode response.

Concluding remarks

Whilst the present article has been necessarily brief, it is hoped that the reader may find the present thread of logic attractive, and may be stimulated to try out ISEs for improving his or her own fermentations. It is useful to list once again the elements of my argument:

1. The entire field of microbial physiology, including that concerned with the production of substances of economic interest, is dominated by the understanding that microbial metabolism is markedly affected by the chemical environment of the culture.
2. Just as most experimenters monitor and control the pH and dissolved oxygen level of their cultures, as this has in the past been found to be useful and economic, so too does the availability of numerous ion-selective electrodes allow the measurement and control of the solution concentration of many other species in real time and on a continuous basis.
3. The ability to optimise the addition of nutrients to microbial cultures, as well as optimising the specific production of fermentation chemicals desired, also avoids waste of expensive feedstock chemicals by obviating their unnecessary consumption.
4. Advances in instrumentation, particularly the availability of microprocessor-based digital electronic systems, now allow sophisticated automatic control systems, in which ISEs may constitute sensing elements, for a very modest investment, relative to the improvement in fermentaion process economics which they can provide.

REFERENCES

1. Pirt, S. J., Principles of microbe and cell cultivation. 1975, 146, Blackwell Scientific, Oxford.
2. Cooney, C. L., *Process Biochem.*, 1979, *14*(5), 31.
3. Finn, R. K. and Fiechter, A., Symp. Soc. Gen. Microbiol., 1979, *29*, 83.
4. Gale, E. F. and Epps, H. M. R., *Biochem. J.*, 1942, *36*, 600.
5. Luedeking, R. and Piret, E. L., *J. Biochem. Microbiol. Technol. Eng.*, 1959, *1*, 393.
6. Luedeking, R. and Piret, E. L., *J. Biochem. Microbiol. Technol. Eng.*, 1959, *1*, 431.
7. Thauer, R. K., *et al., Bacteriol. Rev.* 1977, *41*, 100.
8. Harold, F. M., *Curr. Top. Bioenerg.*, 1977, *6*, 83.
9. Neijssel, O. M. and Tempest, D. W., Symp. Soc. Gen. Microbiol., 1979, *29*, 53.
10. Brookes, R., *Process Biochem.*, 1969, *4*(3), 27.
11. Wimpenny, J. W. T., Symp. Soc. Gen. Microbiol., 1969, *19*, 161.
12. Harrison, D. E. F., *J. Appl. Chem. Biotechnol.*, 1972, *22*, 417.
13. Mukhpadhyay, S. W. and Ghose, T. K., *Process Biochem.*, 1976, *11*, 19; 40.
14. Harrison, D. E. P., The oxygen metabolism of microorganisms, 1976, Meadowfield Press, Durham.
15. Veldkamp, H., Continuous culture in microbial physiology and ecology, 1976, Meadowfield Press, Durham.
16. Flickinger, D. C. and Perlman, D., *Appl. Env. Microbiol.*, 1974, *33*, 706.
17. Rogers, P. J. and Morris, C. A., *Arch. Microbiol.*, 1978, *119*, 323.
18. Hunter, K. and Rose, A. H., *J. Appl. Chem. Biotechnol.*, 1972, *22*, 527.
19. Cysewski, G. R. and Wilke, C. R., *Biotechnol. Bioeng.* 1977, *19*, 1125.
20. Schultz, J. S. and Gerhardt, P., *Bacteriol. Rev.*, 1969, *33*, 1.
21. Kjaergaard, L., *Adv. Biochem. Eng.*, 1977, *7*, 131.
22. D'Ans, G., *et al., Automatica.*, 1972, *8*, 729.
23. Bray, G. A. and Campfield, L. A., *Metabolism.*, 1975, *24*, 99.
24. Calow, P., Biological machines. A cybernetic approach to life. 1976, Edward Arnold, London.
25. Munro, A. L. S., *Meth. Microbiol.*, 1970, *2*, 39.
26. Moody, G. J. and Thomas, J. D. R., Selective ion-sensitive electrodes. 1971, Merrow, Watford.
27. Bailey, P. L., Analysis with ion-selective electrodes. 1976, Heyden, London.
28. Midgley, D. and Torrance, K., Potentiometric water analysis. 1978, John Wiley, Chichester.
29. Vesely, J., *et al.*, Analysis with ion-selective electrodes. 1978, Ellis Horwood, Chichester.
30. Koryta, J., Ion-selective electrodes. 1975, Cambridge University Press, Cambridge.
31. Lakshminarayaniah, N., Membrane electrodes. 1976, Academic Press, London.
32. Baiulescu, G. E. and Cosofret, V. V., Applications of ion-selective membrane electrodes in organic analysis. 1977, Ellis Horwood, Chichester.
33. Buck, R. P., in Freiser, H. (ed) Ion-selective electrodes in analytical chemistry. 1978, 1. Plenum Press, New York.
34. Kell, D. B., *et al., FEBS Lett.*, 1978, *86*, 294.
35. Kjaergaard, L., *Biotechnol. Bioeng.*, 1976, *18*, 285.
36. Nyiri, L. K., *Dev. Ind. Microbiol.*, 1971, *13*, 136.
37. Humphrey, A. E., *Dev. Ind. Microbiol.*, 1977, *18*, 58.
38. Wang, H. Y., *et al., Biotechnol. Bioeng.*, 1977, *19*, 69.
39. Swartz, J. W. and Cooney, C. C., *Process Biochem.*, 1978, *13*(2), 3; 24.
40. Jefferis, R. B., (ed) Workshop — Computer applications in fermentation technology. 1976, Verlag Chemie.
41. Unden, A., *et al., Process. Biochem.*, 1979, *14*(3), 8.
42. Tempest, D. W., *Adv. Microbiol. Physiol.*, 1970, *4*, 223.
43. Stouthamer, A. H., Yield studies in microorganisms. 1976, Meadowfield Press, Durham.
44. Degn, H. and Harrison, D. E. F., *J. Theoret. Biol.*, 1969, *22*, 238.
45. Ishizaki, A., *et al., Agr. Biol. Chem.*, 1973, *37*, 99.
46. Golueke, C. G. and Oswald, W. J., *Ann. Rev. Pl. Physiol.*, 1964, *15*, 387.

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Cata-list . . .

For copies of catalogues listed simply phone the contact given below. Overseas readers only should circle the appropriate numbers on the reader enquiry cards.

A new leaflet published by Data Laboratories Ltd. (Datalab) gives a comprehensive description of DL 2000/SUP Superface. This is a universal interface based on a microprocessor which allows the digital records of waveforms stored in up to 30 channel memories of a DL 2000 series transient recorder to be transferred to virtually any type of programmable calculator, computer, tape punch, or other digital data processing equipment. It can readily and quickly be matched to different devices.

The six user selectable options which provide a wide choice of data codes and formats are summarised. The leaflet contains general information about parallel interfaces, series interfaces, the ASC11 coded format, and the three output buffer versions that are available.

Mr. R. Widenka — 01-640 5321

reader enquiry 90

Brannan Thermometers have produced a new general thermometer catalogue, BT 4.1. From the striking illustration on the front cover, right through its sixteen pages of full colour, the catalogue shows a comprehensive range of thermometers.

The front index lists basic applications and user markets from banana curing to photography.

Nicholas Austin — 0946 810413

reader enquiry 91

Some important findings in the fields of enzyme substrates and polymeric bi-organic reagents, are examined in an article in the first issue of the new Digest published by Koch-Light Laboratories Ltd by Professor S.A. Barker of the University of Birmingham Chemistry Department.

"New developments in fluorimetric enzyme assays" is the second major article in Koch-Light Digest. Written by D.H. Leaback, Biochemistry Department, Institute of Orthopaedics, Stanmore, Middlesex, the article includes reference to a procedure entailing the accurate metering of microlitre volumes of substrates, buffer and enzyme solutions into a micro-reaction chamber using a precision syringe.

Other features in Koch-Light Digest examine hazard codes with the emphasis on 'user' hazards as distinct from transport hazards; the latest guidelines to purchasing poisons; news from the Koch-Light scintillator division and also of biochemical products from Sankyo Co. Limited of Japan.

Dr. P.B. Koch — 0440 2436

reader enquiry 92

Bell & Howell have published a new brochure describing their flush diaphragm pressure transducers which provide the facility of introducing pressure transducers into systems without adding 'dead volume'. This is necessary when either the maximum frequency response is required or the application precludes using transducers having any cavity before the diaphragm. These instruments can be surface mounted using the flange provided.

Robust stainless steel construction provides capability with a wide range of process fluids and available options offer a completely waterproof flying lead electrical connection or a detachable connector.

Transducers are available in pressure ranges from 0-0.75 to 0-100 bar. Types are available for special applications, including crevice free instruments suitable for hygienic applications with a choice of ISS/IDF, Triclover or RJT fittings.

D.J. Maundrell — 0256 20244

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8. Stauber, L. A., *Sci Repts 1st Super Sanita,* 1962, *2,* 68.
9. Goldstein, N. I., *et al., J. Antibiot.,* 1976, *29,* 656.
10. Utahara, R., *et al., J. Antibiot.,* 1954, *7A,* 120.
11. Fisher, P. B., *et al., J. Cellular Physiol.,* 1978, *97,* 345.
12. Bonner, D. P., *et al., J. Antibiot.,* 1972, *25,* 261.
13. Gouge, T. and Andriole, V. T., *J. Lab Clin. Med.,* 1971, *78,* 713.
14. Lechevalier, H., *Antibiot. Chemother.,* 1961, *4,* 640.
15. Mechlinski, W. and Schaffner, C. P., *J. Antibiot.,* 1972, *25,* 256.
16. Howarth, W. R., *et al., Antimicrob. Agents Chemother.,* 1975, *78,* 58.
17. Keim, G. R., Jr, *et al., Science,* 1973, *179,* 584.
18. Fisher, P. B., *et al., Cancer Res.,* 1975, *35,* 1996.
19. Fisher, P. B., *et al., In Vitro,* 1976, *12,* 133.
20. Fisher, P. B., *et al., J. Antibiot.,* 1975, *28,* 896.
21. Goldstein, N. I., *et al., J. Antibiot.,* 1977, *30,* 321.
22. Bonner, D. P., *et al., In Vitro,* 1976, *12,* 399.
23. Fisher, P. B., "Tissue Culture Association Manual-Techniques, Methods and Procedures for Cell, Tissue and Organ Culture" Vol. 2 p. 363. eds. V. J. Evans, V. P. Perry and M. M. Vincent, Tissue Culture Association, Maryland, 1976.
24. Stevens, N. M., *et al., Arch. Virol.,* 1975, *48,* 391.
25. Goldstein, N. I. and Fisher, P. B., "Tissue Culture Association Manual-Techniques, Methods and Procedures for Cell, Tissue and Organ Culture" Vol. 2 p. 281. eds. V. J. Evans, V. P. Perry, and M. M. Vincent, Tissue Culture Association, Maryland, 1976.
26. Goldstein, N. I. and Fisher, P. B., "Tissue Culture Association Manual-Techniques, Methods and Procedures for Cell Tissue and Organ Culture" Vol. 2 p. 341. eds. V. J. Evans, V. P. Perry, and M. M. Vincent, Tissue Culture Association, Maryland, 1976.
27. Fisher, P. B. and Goldstein, N. I., "Tissue Culture Association Manual-Techniques, Methods and Procedures for Cell, Tissue and Organ Culture" Vol. 3 p. 625. eds. V. J. Evans, V. P. Perry, and M. M. Vincent, Tissue Culture Association, Maryland, 1977.
28. Fisher, P. B., *et al., J. Antibiot.,* 1977, *30,* 118.
29. Goldstein, N. I. and Fisher, P. B., *In Vitro,* 1978, *14,* 200.
30. Fisher, P. B., *et al.,* "Tissue Culture Association Manual-Techniques, Methods and Procedures for Cell, Tissue and Organ Culture" Vol 4 p. 805. eds. V. J. Evans, V. P. Perry and M. M. Vincent, Tissue Culture Association, Maryland, 1978.
31. Cass, A., *et al., J. Gen. Physiol.,* 1970, *56,* 100.
32. Kinsky, S. C., *Ann. Rev. Pharmacol.,* 1970, *10,* 119.
33. Strom R., *et al., Biophys. J.,* 1973, *13,* 581.
34. DeKruijff, *et al., Biochim. Biophys. Acta,* 1974, *339,* 30.
35. Fisher, P. B. and Weinstein, I. B., "Carcinogens in Industry and Environment", in press. ed. J. M. Sontag, Marcel Dekker Press, New York, 1980.
36. De Kruijff, B. and Demel, R. A., *Biochim. Boiphys. Acta,* 1974, *339,* 57.
37. Zygmunt, W. A. and Tavormina, P. A., *Appl. Microbiol.,* 1966, *14,* 865.
38. Schaffner, C. P. and Mechlinski, W., *J. Antibiot.,* 1972, *25,* 259.
39. Fisher, P. B. and Bryson, V., *In Vitro,* 1977, *13,* 548.
40. Fisher, P. B., *et al., J. Cell Sci.,* 1978, *32,* 433.
41. Mechlinski, W., Personal communication.
42. Lampen, J. O., *Symp. Soc. Gen. Microbiol.,* 1966, *16,* 111.
43. Fisher, P. B. and Bryson, V., *In Vitro,* 1976, *12,* 324.
44. Fisher, P. B., *et al., J. Antibiot.,* 1979, *32,* 301.
45. Amati, P. and Lago, C., *Nature,* 1974, *247,* 466.
46. Fisher, P. B. *et al., J. Cellular Physiol,* 1979, *100,* 335
47. Fisher, P. B., *et al., In Vitro,* 1974, *10,* 339.
48. Phillips, B. J. and Ambrose, E. J., *Nature New Biol.,* 1971, *231,* 146.
49. Harris, M., *J. Natl. Cancer Inst.,* 1973, *50,* 423.
50. Fisher, P. B., *et al., Proc. Soc. Exp. Biol. Med.,* 1974, *146,* 1122.
51. Hidaka, H., *et al., Cell,* 1978, *14,* 415.
52. Perlman, D. and Brindle, S. A., *Antimicrob. Agents Chemother.,* 1963, *3,* 458.
53. Hemphill, J. J., *et al.,* "Antibiotics Annual, 1957-1958" p. 961. eds. H. Welch, and F. Marti-Ibanez, Medical Encylopedia Inc., New York, 1958.
54. Perlman, D., *et al., Appl. Microbiol.,* 1967, *15,* 82.
55. Pollock, M. E., *et al., Proc. Soc. Exp. Biol. Med.,* 1960, *105,* 10.
56. Scriba, M., *Med. Mikrobiol. Immunol.,* 1968, *154,* 267.

The Multidisc-T: A Helpful Aid for the Miniaturisation of Various Diagnostic Microbiological Techniques

continued from page 26

19. Oxoid Deutschland GmbH 4230 Wesel 1. West Germany.
20. Sharpe, J. E., *et al.,* Rapid methods and automation in Microbiology p. 63. eds. H. H. Johnston, S. W. B. Newsom. Learned Information Ltd. Oxford — New York, 1976.
21. Postgate, J. R. Methods in Microbiology. Vol.1 p.611. eds. J. R. Norris, D. W. Ribbons. Academic Press. London — New York, 1969.
22. Malik, K. A., Zbl. Bakt. Hyg., I. Abt. Orig. A., 1977, *237,* 415.
24. Holliday, R., *Nature,* 1956, *178,* 987.
24. Delafield, F. P., *et al., J. Bacteriol.* 1965, *90,* 1455.
25. Stanier, R. Y., *et al., J. Gen. Microbiol.,* 1966, *43,* 159.
26. Malik, K. A. and Claus, D., *J. of Appl. Bacteriol.,* 1977, *45,* 143.
27. Fusaro, R. M., *Appl. Microbiol.,* 1972, *23,* 174.
28. Kurtzman, R. H., *Mycologica,* 1973, *65,* 236.
29. Mic-200-96- Channel dispenser. Dyonatech. Labs. Ltd. U.K.
30. Miniteck — Becton, Dickinson. Ltd. U.K.
31. Titertek, Multidiluter, Flow Labs. Ltd, U.K.
32. Burger, H. and Quest, R., New approaches to the identification of microorganisms. p. 39. eds. C. G. Heden, T. Illeni. John Wiley and Sons. London — New York, 1974.

The Role of Ion-Selective Electrodes in Improving Fermentation Yields

continued from page 23

47. Benemann, J. R., *et al., Nature.,* 1977, *268,* 19.
48. Mosey, F. E., *et al.,* Water Poll. Conf., 1971, *6,* 1.
49. Mikami, E. and Misono, T., *J. Ferment. Technol.,* 1968, *46,* 1056.
50. John, P., *J. Gen. Microbiol.,* 1977, *98,* 231.
51. Tuovinen, O. H. and Nicholas, D. J. D., *Appl. Env. Microbiol.,* 1977, *33,* 477.
52. Cutler, S. G., *et al., J. Electroanal. Chem.,* 1977, *85,* 145.
53. Schwartz, W., Conference Bacterial Leaching. 1977, Verlag Chemie.
54. Bosecker, K. and Kursten, M., *Process. Biochem.,* 1978, *13*(10), 2.
55. Kelly, D. P., *et al.,* Symp. Soc. Gen. Microbiol., 1979, *29,* 263.
56. Weinberg. E. D., *Dev. Ind. Microbiol.,* 1974, *15,* 70.
57. Updike, S. J. and Hicks, G. P., *Nature.,* 1967, *214,* 986.
58. Nilsson. H., *et al., Biochem. Biophys. Acta.,* 1973, *320,* 529.
59. Cullen, L. F., *et al., Analyt. Chem.,* 1974, *46,* 1955.
60. Barker, S. A. and Somers, P. J., in Wiseman, A. (ed) Topics in Enzyme and Fermentation Biotechnology. 1978, *2,* 120. Ellis Horwood, Chichester.
61. Camman, K., *Z. Anal. Chem.,* 1977, *287,* 1.
62. Enfors, S-O. and Dostalek, M., *Process Biochem.,* 1975, *10,* 6, 13.