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Review Article

The control and measurement of 'CO_2' during fermentations

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1. Introduction

It has been known for many years that the growth and metabolism of microorganisms is accompanied by the uptake and/or evolution of CO_2. To study and indeed to exploit the effects of CO_2 on microbial metabolism, it is necessary to control the level of (dissolved) CO_2 within the culture medium. Whilst several articles cover one or two methods by which pCO_2 may be measured and, hence, controlled, we know of no review of these. Thus, the purpose of the present article is to provide an overview of the role, measurement and control of the magnitude of pCO_2 during laboratory and industrial fermentations. We begin by describing the various 'CO_2' equilibria and the question of CO_2-absorption rates.

2. 'CO_2' Concentrations

Although the partial pressure of CO_2 in the gas phase may be held constant, the ratios of the different possible species of 'CO_2' in the aqueous phase will vary as a function of the pH and other factors. Since CO_2 can hydrate and dissociate in water, the reaction scheme may be written [1] as:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+ \quad (1)$$

In addition, it has recently been proposed that small concentrations of dimeric hydrogen carbonate ions ($H_3C_2O_6^-$) exist near neutral pH [2]. Since the concentration of this species is negligible, however, such ions will not be considered in the following. At pH values of < 8, the concentration of carbonate ions may be neglected [3] and only the following hydration reactions need to be considered:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \cdot \quad (2)$$

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2.1. Dissolved CO_2 concentration

The concentration of CO_2 in solution ($[CO_2]_{aq}$) is normally expressed by Henry's law [4]:

$$[CO_2]_{aq} = K_H pCO_2 \tag{3}$$

where K_H = Henry's law constant ($mol \cdot atm^{-1}$) and pCO_2 = the partial pressure of CO_2 in the gas phase (atm). For cultures grown under atmospheric pressure, the proportionality of solubility and partial pressures (Henry's law) may be assumed without introducing appreciable errors [5].

At a temperature of 37 °C, $K_H = 10^{-1.61}$ [4] where the $[CO_2]$ is expressed in molar terms. Thus, to obtain $[CO_2]$ in millimolar terms, $K_H = 10^{1.39}$. Hence:

$$[CO_2]_{aq} = 10^{1.39} \times pCO_2 = 24.6 \times pCO_2.$$

In other words, when $pCO_2 = 1$ atm, the concentration of dissolved $CO_2 = 24.6$ $mmol \cdot l^{-1}$.

2.2. Bicarbonate concentration in a pH-controlled culture with a constant temperature and gas phase

The equilibrium between CO_2 and HCO_3^- is defined by a 'hybrid' equilibrium constant K_1' [4] where:

$$K_1' = \frac{10^{-pH} \cdot [HCO_3^-]}{[CO_2]} \tag{4}$$

From Eqn. 4, it follows that:

$$\log[HCO_3^-] = pH - pK_1' + \log[CO_2] \cdot \tag{5}$$

pK_1' is related to the thermodynamic pK of the reaction $pK_1°$ and the ionic strength I by:

$$pK_1' = pK_1° - 0.5f(I) - bI. \tag{6}$$

From Davies's Eqn. 4:

$$f(I) = [I^{1/2}/(1+I^{1/2}) - 0.21][298/(T+273)]^{2/3} \tag{7}$$

where T is the temperature in °C. I is the ionic strength of the medium and is given by [4, 6]:

$$I = \tfrac{1}{2}\ \Sigma c_i z_i^2 \tag{8}$$

where c_i = the concentration of ion i and z_i = the charge on ion i.

To obtain the apparent $pK_{a,1}$ for the CO_2/HCO_3^- equilibrium, we use [4]:

$$pK_{a,1} = pK^{\circ}_{a,1} - f(I) - bI \tag{9}$$

Therefore, from Eqn. 9, we obtain $pK_{a,1}$, and so from Eqn. 5:

$$\log[HCO_3^-] = pH - pK_{a,1} + \log[CO_2].$$

It may be noted that these equations assume that there is an equilibrium between the pCO_2 in the gas phase and that in solution. Clearly, this implies that the rate of exchange of CO_2 between these two phases is rapid. It is, therefore, pertinent to determine the extent to which this is so.

3. Rates of Exchange of CO_2 between Fermentor Broths and Gas Phase

Following a step increase in the pCO_2 in the gas phase, the rate of gas absorption $(R_s) \cdot \text{vol}^{-1}$ of liquid is given [7] by:

$$R_s = \frac{dc}{dt} = \frac{k_L a}{h}(c_s - c) \tag{10}$$

where $c_s =$ gas concentration at the interface, $c =$ concentration of the gas in the bulk liquid, $a =$ interfacial area, $k_L =$ a constant dependent upon the diffusion coefficient of the gas and $h =$ the thickness of the stationary film.

In general, because the value of h is not known, it is usual to combine it with k_L and write [6, 7]:

$$R_s = K_L a(c_s - c). \tag{11}$$

The absorption rate can be expressed in terms of partia pressures»in gas and liquid (p_g and p_l, respectively) by making the substitutions $c_s = Hp_g$ and $c = Hp_l$ (where H = Henry's law constant) into Eqn. 11:

$$R_s = K_L aH(p_g - P_l). \tag{12}$$

The maximum absorption rate for a given partial pressure occurs when $c = 0$ and, hence, $Hp_l = 0$. Then:

$$R_{s,max} = K_L a c_s = K_L a H p_g. \tag{13}$$

The rate of absorption of CO_2 is given by the slope of plots of 'CO_2' concentration vs. time. From Eqn. 13, it can be seen that:

$$K_L a = R_s / Hp_g.$$

At 37 °C, $H = 10^{-1.61}$ when ['CO_2'] is expressed in M [4] and $H = 10^{1.39}$ when ['CO_2'] is expressed in mM. Hence:

$$K_L a = R_s / 10^{1.39} \cdot p_g.$$

For a detailed review of the diffusion, desorption and solubility of CO_2, in addition to some of the reactions involving CO_2, see also Ho et al. [6]. The rate of diffusion of CO_2 through a silicone membrane may also be used to measure pCO_2 [7a]. Having outlined CO_2-absorption rates and equilibria, we will now discuss various methods by which CO_2 may be estimated (Table 1).

4. Traditional Methods for Estimation of CO_2

The detection of CO_2 in cultures includes both qualitative and quantitative methods. Production of CO_2 from various carbohydrate substrata is a useful diagnostic tool in the identification of bacteria. Several methods for detecting CO_2 production have been used, e.g., Durham tubes inserted into broth cultures [8], displacement of agar plugs [8, 9] and the appearance of cracks in agar medium [10]. The Eldredge tube [11], which is a device for trapping CO_2 in barium hydroxide, can be used for both the qualitative (observation of a precipitate) and quantitative (recovery of the

TABLE 1

METHODS USED IN DETERMINATION OF CO_2. METHODS ARE LISTED IN THE ORDER THAT THEY APPEAR IN TEXT

Method	Principle	Selected references
Titrimetric	A standard alkaline solution is neutralised after absorbing CO_2	20
Colourimetric	Modification of colour of an indicator solution	21, 22
Gravimetric	Isolation and weighing of a compound of CO_2 (e.g., barium carbonate)	12, 20
Volumetric (Orsat)	Decrease in volume of a gas sample after chemical removal of CO_2	27
Volumetric (Warburg)	Increase headspace volume above an acidified sample	31
Manometric	Increase in pressure of headspace above an acidified sample	31
Katharometer	Differences in thermal conductivities of gases	27
Mass spectroscopy	Differences in mass to charge ratios of ions	35
IR	Absorption of IR radiation	27
Fibre optic probes	Changes in light transmittance of pH indicator	48
Chromatography	Differences in column retention times of gases	36
Potentiometric	Changes in pH induced by CO_2	50
Conductiometric	Changes in water conductivity caused by CO_2	70, 71
Amperometric	Flow of electric current due to reduction of CO_2	75
Piezoelectric	Change in resonant frequency due to adsorption of CO_2	103

precipitate) detection of CO_2 [12]. However, these procedures depend upon the evolution of gaseous CO_2 from the growth medium which does not always occur due to the high solubility of CO_2 in water. The insertion of a red-hot loop into cultures resulted in a copious evolution of gas [13, 14] and this method of CO_2 detection is known as the hot-loop method. Displacement of agar plugs is used in the hot-tube method which is more sensitive than the hot-loop method [15]. In the hot-tube method, an agar plug is displaced by the evolution of gaseous CO_2 when the culture is heated to 80 °C.

In the estimation of CO_2 by the Conway microdiffusion method [16, 17], CO_2 is liberated by acidifying the sample. There have been a number of designs of the apparatus for the microdiffusion method [16–18] but they are all based upon the standard cell illustrated in Fig.1. CO_2, liberated from the sample in the outer compartment, is allowed to diffuse to the central compartment where it is absorbed by a standard barium hydroxide solution [KOH or NaOH may be used instead of $Ba(OH)_2$] containing thymolphthalein indicator. The contents of the inner compartment are then titrated with a standard hydrochloric acid until the thymolphthalein indicator is just colourless (pH $\approx$ 9.3) at which point the excess of barium hydroxide is neutralised [16, 17]. CO_2 in a liquid sample may be estimated directly. Phenolphthalein is added to the sample which is then titrated with a standard sodium hydroxide solution. Free CO_2 reacts with the sodium hydroxide to form sodium bicarbonate. Completion of the reaction is indicated by the development of the pink colour characteristic of phenolphthalein at the equivalence point of pH 8.3 [19]. Titrimetric methods may also be used for the determination of CO_2 in gases. The CO_2 in the gas sample is absorbed into a standard barium hydroxide solution which, after the addition of phenolphthalein indicator, is titrated with standard hydrochloric acid [20].

Colourimetric methods, for the estimation of the CO_2 concentration in a gas, do not require titration of the CO_2-absorbing solution. The colour intensity of a solution of phenolphthalein's sodium salt decreases as the concentration of CO_2 increases [21, 22]. Similarly, the concentration of CO_2 may be estimated by the modification of the colour of alizarin yellow R in the presence of NaOH [22]. CO_2-detection tubes are also based on a colour change caused by a chemical reaction between the gas and tube

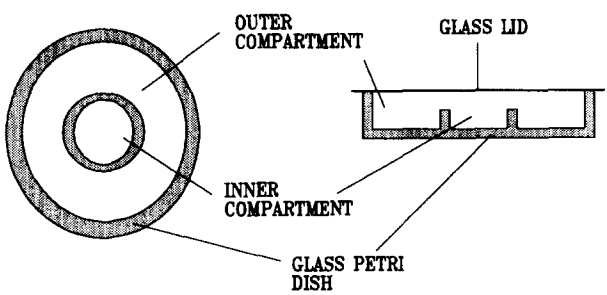

Fig. 1. Conway's standard microdiffusion cell. The cell consists of a Petri dish in the centre of which is fused a piece of glass tubing to form an inner cell or compartment. The edge of the outer dish is ground so that a lid, in the form of a square of flat ground glass, may enclose completely any gases within the dish. A mixture of vaseline and paraffin is smeared onto the lid to assist the seal.

contents [23]. When a gas containing CO_2 is brought into equilibrium with sodium bicarbonate solution, the pH is a measure of the concentration of CO_2 [24]. The pH may then be determined by the use of indicators [25] or with a pH electrode (see also Section 9.1.1.). When combined with a spectrophotometer the pH, and hence pCO_2, of a sodium bicarbonate solution, containing indicator and sparged with a CO_2-containing gas, may be estimated on a continuous basis [25].

Other traditional methods of measuring CO_2 involve the passage of a measured stream of gas through absorbants followed by gravimetric [e.g., 12] or volumetric measurements [26, 27]. Such a method was employed in the Orsat apparatus in which the change in volume, at constant pressure, of a gaseous sample, following the removal of CO_2 by its absorption into KOH, was measured and corresponded to the pCO_2 [26, 27].

Total 'CO_2' may be measured by first saturating a sample with CO_2, then acidifying the sample and measuring either the change in the volume of the headspace at a constant pressure or the change in the pressure of the headspace at a constant volume [26, 28–32]. The latter method does not require gas meters for the accurate measurement of volume, and an example of an apparatus for such a method is shown in Fig. 2. Interference from volatile acids, which become gaseous in acidic conditions, is a possibility; however, interference due to organic or inorganic compounds commonly found in fermentation liquors are either negligible or easily eliminated [31]. Although such a method requires only very simple instrumentation and may easily be automated, it is not able to provide genuinely continuous monitoring of the total 'CO_2'.

Enzymatic methods for the estimation of various compounds are specific for the compound(s) involved. One such method exists for the estimation of 'CO_2' and is commercially availabe (e.g., from the Sigma Chemical Company). The method is based upon the reaction catalysed by phosphoenolpyruvate carboxylase which produces oxaloacetate from phosphoenolpyruvate and 'CO_2' [33]. Malate dehydrogenase then

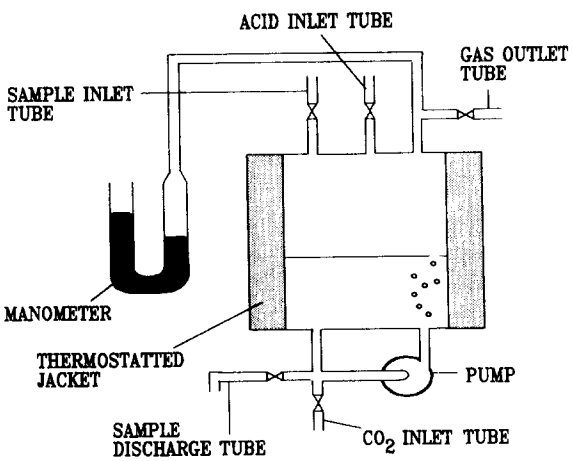

Fig. 2. Design of an apparatus for manometric determination of CO_2. CO_2 is estimated by measuring the increase in pressure of the headspace following acidification of sample [31].

converts NADH and the so-formed oxaloacetate to malate and NAD^+. When the absorbance is read, at either 340 or 380 nm, the decrease in absorbance upon the addition of PEP is proportional to the original 'CO_2' content of the sample [33].

With the exception of the colourimetric method, the 'traditional' methods, discussed above, can only be used for spot checks and are therefore not suitable for continuous analysis. Methods employed in the gas analysis of clinical samples (e.g., blood) are also unsuitable for continuous use, since they are performed on samples taken from a patient [e.g., 32, 34].

5. CO_2 Analysis by Katharometer

The principle of CO_2 analysis by katharometer [27] is as described below. The behaviour of the katharometer reflects the thermal conductivity of the gas (mixture) which is passing through it. Within the katharometer are four small cells, each containing a glass-coated Pt wire identical with the others. These four wires form the arms of a Wheatstone bridge (Fig. 3). Two of the cells (B, D) are exposed to a reference gas and the other two (A, C) are exposed to the sample gas. When the bridge current is constant and all four cells are exposed to the same gas, each wire will attain the same temperature and resistance. Under these conditions, the bridge is balanced and no current flows through the galvanometer.

If two gases of different thermal conductivities, such as air and an air/CO_2 mixture, are introduced into the cells (air into the reference cells and the mixture into the sample cells), then Wires B and D will lose more heat than will Wires A and C. This is because the thermal conductivity of air is greater than that of CO_2. The consequent change in the conductivities of the wires will unbalance the bridge and cause a deflection of the galvanometer, with the size of the deflection being dependent upon the difference in the thermal conductivity, and in this instance pCO_2, of the two gases. However, the measurement of gases by katharometers is not specific, e.g., for CO_2. To measure the pCO_2 of the effluent gas from a fermentor the constituent gases

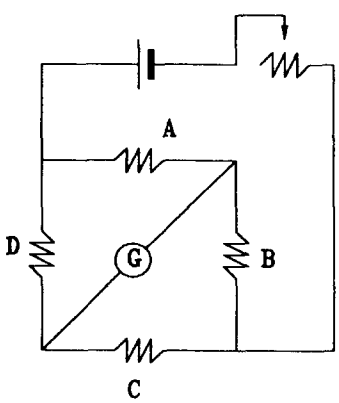

Fig. 3. A simplified representation of a katharometer. Sample and reference cells each contain a Pt wire (A–D), each of which is identical, forming the arms of a Wheatstone bridge. The principle of CO_2 measurement using a katharometer is as described in text.

would first have to be separated, for example, by gas chromatography as described in Section 8.

6. Mass Spectrometry

The basic principle of mass spectrometry is the separation and registration of ionic masses [35]. The sample is ionised, e.g., by electron bombardment, and the ions, with a mass-to-charge ratio of m/z, are accelerated by an electric field through a series of slits to form an ion beam. This ion beam is then deflected by a magnetic field, H, according to $m/z=(H^2r^2)/2V$ where H is the strength of the magnetic field, r the curvature of the pathway (instrumentally dictated) and V the voltage used to accelerate the ions [35]. By continuous scanning of either the magnetic field or the accelerating voltage, ions of different m/z values can be focussed on the detector and subsequently measured. In addition to the identification of compounds, the mass spectrometer can be adapted for selected ion monitoring to permit highly selective determinations of very high sensitivity [36]. However, mass spectrometry cannot always be used to examine a number of substances simultaneously without additional separation techniques such as gas chromatography (see Section 1.5). The reason for this is that mass spectra tend to be complicated and examination of several compounds simultaneously can lead to significant overlap, seriously compromising the quantitation of the individual species [37]. Despite this problem, gases produced by fermentation processes can, in fact, be estimated simultaneously by mass spectrometry [38–40], by exploiting the fact that different gases have different isotopic compositions. CO_2 is measured at $m/z=44$. The only possible interference is from N_2O (formed by many denitrifiers) and from volatile fatty acids (which may break down to CO_2 within the mass spectrometer [39]).

The type of mass spectrometer described above is the magnetic mass spectrometer which is perhaps more often used for the analysis of fermentor exhaust gases whilst quadrupole mass spectrometers are used for the estimation of dissolved gases [39]. These nonmagnetic mass spectrometers employ as a mass filter four electrically conducting parallel rods precisely located in a ceramic holder. The opposite rods are connected electrically and a radio-frequency (*RF*) voltage superimposed on a *DC* voltage is applied to the two pairs of rods, 180° out of phase with each other [35, 38–40]. Ions entering at one end of this array are constrained to make oscillations in the transverse electric field. At a given *RF/DC* voltage, only ions of a specific m/z value will avoid collision with one of the rods and will, thus, pass through the filter. Scanning the *RF* and *DC* voltages may be performed rapidly with the ions being transmitted sequentially in order of their m/z ratios with constant resolution [35, 38–40].

Sampling dissolved gases is achieved by a membrane inlet to the mass spectrometer [39–41]. A perforated capillary or sheet, or porous material, is covered with a plastic membrane and immersed into the fermentation medium. A vacuum is applied to the inside of the probe and the dissolved gases diffuse through the membrane. Since the diffusion coefficient is dependent upon both the gas and the membrane used, the membrane inlet is selective. Another means by which the dissolved gases in fermentation media could be sampled is by a carrier gas tubing method [3, 42]. This method involves passing a carrier gas through a tube, or across a membrane, made of a gas-

porous material, immersed in the fermentation medium. The exhaust gas from the tubing is then analysed.

Unfortunately, methods such as mass spectrometry and infrared analyses (see the following section) are normally prohibitively expensive for routine use on single fermentors at the laboratory scale [43].

7. Optical Methods of CO_2 Determination

7.1. IR absorbance

The absorbance of IR radiation by a gas (mixture) is proportional to the mass of absorbing molecules in the light path and is, therefore, proportional to the absolute pressure of the gas in an analysis cell [27]. The principle of IR spectroscopy for estimating the partial pressures of gases is as follows [27, 44]. When analysing a single component in a gas stream, a so-called 'nondispersive analyser' is used. This examines absorbance in the wavelength range of $3-15$ μm. The light beam, after passing through the absorbance cell through which the gas is flowing, passes to a detector that is selective for the wavelength at which the component being analysed absorbs. The intensity of the transmitted light is compared with the intensity of the incident light by using a 'blank' or reference cell filled with CO_2-free gas. The difference between the two signals is a measure of the pCO_2. In a null-balance instrument, this difference is used to actuate a servo system. This adjusts a shutter situated in the light path of the reference cell until the sample and reference radiations are matched. The shutter position is a measure of the pCO_2.

Water absorbs strongly in the IR [45] and, hence, the presence of water vapour in the gas sample may interfere with the IR spectrophotometric measurement of CO_2. Since this absorption is characteristic of $-OH$ bonds, volatile alcohols may also interfere. Some other compounds from bacterial cultures, which absorb in the IR and those may, thus, interfere, are acetone, acetaldehyde and substances with $-SH$ bonds [44]. IR gas analysis is, therefore, not selective for CO_2 unless the gas sample has been treated to remove interfering substances prior to the pCO_2 measurement and, as stated above, this technique may be rather expensive.

7.2. Fibre optic measurements

Stimulated by recent developments in fibre optics technology, a number of new uses of fibre optics in sensing have been proposed. The development of fibre optic pH probes [46] has led to the production of fibre optic CO_2 probes [47, 48]. In such fibre optic CO_2 probes, ambient pCO_2 controls the pH of a bicarbonate buffer solution, contained within a membrane, which then influences the optical transmittance of a colourimetric pH indicator. One fibre carries light to the buffer/indicator solution, and a second fibre carries the transmitted signal (Fig. 4) to a receiver which converts the signal to an electrical output. The optical approach avoids problems of making miniature glass or liquid membrane pCO_2 electrodes [49] with adequate reference electrode stability. However, the changes in pH, that are measured with such a device, are not necessarily due solely to a change in $pCO_{2,aq}$ since organic acids or bases that are able to penetrate the membrane may also cause such a change in pH [50, 51].

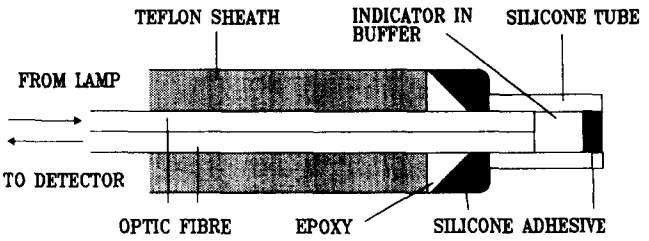

Fig. 4. Construction of sensor tip of a fibre optic probe. Two fibres extending from end of appropriately opaque and inert tubing are sealed into it with epoxy. Epoxy is also layered onto cut end of fibres to prevent binding of indicator dye. Clear silicone adhesive is coated over epoxy except at fibre ends. A piece of silicone tubing is then plugged at one end with white silicone adhesive and filled with a solution of phenol red, $KHCO_3$ and KCl [48].

8. Chromatographic CO_2 Determination

In gas liquid chromatography, a sample is introduced into a stream of carrier gas (the mobile phase) and is swept through a chromatographic column which contains a stationary phase. After introduction onto the column, the sample components are allowed to distribute between the stationary and mobile phases according to their partition coefficients. K, the partition coefficient, is defined [36, 52] as:

$$K = C_s/C_m$$

where C_s is the concentration of solute in the stationary phase and C_m is the concentration of solute in the mobile phase. The lower the value of K, i.e., the lower the solubility of the solute in the stationary phase, the quicker it will pass through the column. Various components of a sample can be separated only if they have different K values. The time taken to pass through a column, the retention time, will be affected by the type of column, temperature and carrier-gas flow rate. Once separated, the components enter a detector (e.g., thermal conductivity detector, mass spectrometer) connected to a recorder or a printer/plotter and are consecutively registered as chromatographic peaks. Provided that the detector and separation procedure are appropriate, each peak will correspond to one of the components of the original sample. An internal standard may be added to the sample before analysis to compensate for possible variations in analytical conditions. The retention times of the components are then calculated relative to that of the internal standard. For a more detailed discussion of chromatographic separation theory, see, e.g., Larsson and Odham [36], Perry [52], Grob [53], Cramers and McNair [54] and Willett [55]. The area beneath each peak can then be correlated to the concentration of the corresponding component in the injected sample [36, 52, 54, 55]. However, since chromatographic techniques rely upon the spatial separation of the compounds that are being quantified, they are only of use on a noncontinuous basis.

9. CO_2 Electrodes

9.1. Potentiometric

Ion-selective electrodes may be utilised for the estimation of a wide range of compounds, including gases, e.g., ammonia, O_2 and CO_2 [56–58]. The determination of $pCO_{2,aq}$ by ion-selective potentiometric electrodes, such as the Severinghaus-type (Fig. 5), involves the measurement of the pH of a thin layer of a buffer solution in equilibrium with ambient CO_2 [57, 59]. The fermentation medium and the buffer solution within the electrode are separated by a membrane which is 'freely' permeable to CO_2. However, the use of the Severinghaus-type potentiometric electrode for measuring the levels of dissolved CO_2 is hampered by its slow response time; complex relationships among multiple chemical species during unsteady diffusion were shown to cause hysteresis and pH-dependent response rates [59]. Digital simulations may be used to predict the dynamic response of such electrodes [60] and electrode stability may be sacrificed to improve response time. As with the fibre optic probes mentioned above, the changes in pH that are measured with such a device are not necessarily due solely to a change in $pCO_{2,aq}$ [50]; additionally, the electrodes are by no means cheap.

Another problem with devices, such as the Severinghaus-type electrode, is that they must be immersed in the fermentation broth and are, therefore, 'invasive'. The use of such invasive devices leads to a number of potential problems: an additional port on the fermentation vessel will be necessary leading to an increased risk of contamination: the device must be robust enough to withstand sterilisation: the device is required to be calibrated easily without having to be removed from the fermentation vessel. Such a steam-sterilizable Severinghaus-type pCO_2 electrode that can be calibrated in situ, has, in fact, been described [61].

K_2CO_3 may be used as a solid electrolyte for the potentiometric measurement of gaseous carbon oxides. It was shown that the triple contacts: $K_2CO_3(s)$, $CO_2(g)$, Pt(s) and: $K_2CO_3(s)$, $CO_2(g)$, $ZrO_2-CaO(s)$ can be used for CO_2 determination in air- or in O_2-bearing gases at temperatures ranging from 450 to 750 °C [62].

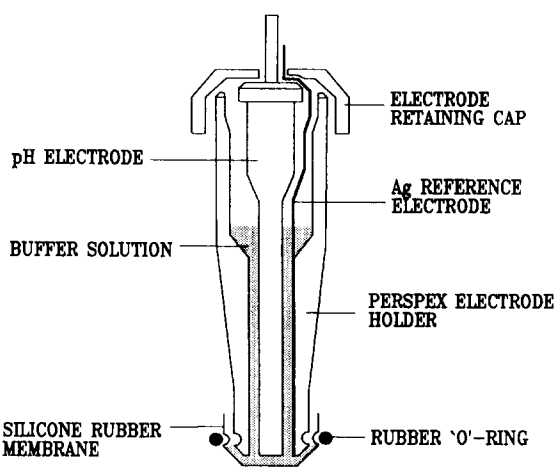

Fig. 5. Design of Severinghaus-type electrode. CO_2 is absorbed into or desorbs from buffer solution and corresponding change in pH is measured by pH electrode [50].

The fabrication and performance of a variety of ion-sensitive field-effect transistors (ISFET), which are in effect solid-state devices (no internal reference electrolyte), have been described [63–65]. The ISFET has attracted considerable interest because it is envisaged that a single miniaturised solid-state chip could contain multiple sensors and be used to sense several ions simultaneously. It has been shown that ISFETs based on ion-selective membranes were subject to positive interference by either CO_2 or organic acids [51]. When field-effect transistors were coated with PVC or silicone rubber membranes, larger responses to CO_2 than with ion-selective membranes were observed and the resulting ISFETs behaved very much like Severinghaus-type potentiometric CO_2 sensors [51]. The response times, however, were much slower than with Severinghaus-type electrodes and the ISFETs still responded to organic acids. ISFETs, like Severinghaus-type electrodes, are invasive devices and, therefore, possess all of the problems associated with invasive devices listed above. However, for gas analysis, rather than the determination of the concentrations of particular ions in a solution, ISFETs could be used as noninvasive devices by simply placing them in the effluent gas flow.

9.1.1. Invasive and noninvasive electrode methods for determination of CO_2. The measurement of pCO_2 in the effluent gas is said to give an excellent approximation of the $pCO_{2,aq}$ [3, 66 but cf. 6], thus, eliminating the need for the more technically demanding measurement of $pCO_{2,aq}$ and allowing the utilisation of noninvasive devices. It is possible, however, to use an invasive device in a noninvasive way. A potentiometric method was proposed whereby changes in pH, caused by the absorption of CO_2 when the effluent gas was bubbled through a solution of sodium carbonate, were measured [23]. This method is still hampered by the problems associated with Severinghaus-type electrodes listed above. Noninvasive methods for determining pCO_2 have received attention for clinical uses in the transcutaneous analysis of arterial pCO_2 [e.g., 67–69]. The principle involved is once again the measurement of the pH change within a buffer solution and, despite the slow response time, such a technique is of use in control systems for maintaining a normal arterial pCO_2 by artificial respiration since such sensors are capable of continuous measurement.

9.2. Conductimetric

Conductimetry has also been applied to the measurement of pCO_2. A continuous conductimetric sensor for CO_2 (Fig. 6) was described in which CO_2 diffused through a hydrophobic gas-porous membrane into a thin layer of pure water [70, 71]. The back wall of the thin water layer (see Fig. 6) was a porous screen separating the water from a mixed-bed ion-exchange column which continuously removed ionic species from the water layer. Electrodes positioned in the water layer continuously measured its conductance. The diffusion of CO_2 into the water layer and the removal of ionic species by the mixed-bed ionic exchanger established a steady-state concentration gradient of CO_2. The gradient was proportional to pCO_2 in the gas phase and the cell conductance was proportional to $(pCO_2)^{1/2}$ [70, 71]. However, this device was evaluated for use in the determination of pCO_2 only up to 0.01 atm.

9.3. Amperometric

In contrast to CO_2, O_2 is routinely and more-or-less reliably measured either

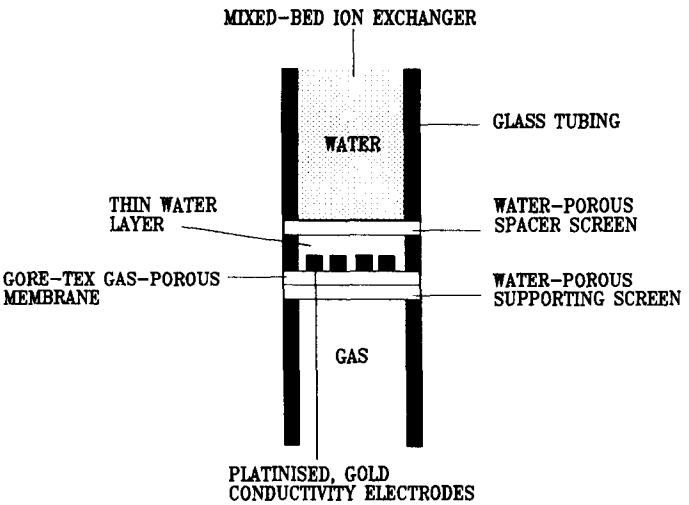

Fig. 6. Schematic representation of a continuous conductimetric CO_2 sensor. Electrodes positioned in thin water layer measure its conductance. Diffusion of CO_2 through the membrane into water layer and removal of ionic species through screen by ion exchanger establish a steady-state gradient of CO_2 [70, 71].

paramagnetically [27] or by means of the Clark-type amperometric electrode [43, 72] which consists typically of an Ag ring anode surrounding a Pt cathode (Fig. 7). When an appropriate potential is applied across the electrodes, O_2 is reduced at the cathode in the reaction:

$$2H^+ + 2e^- + \tfrac{1}{2}O_2 \rightarrow H_2O.$$

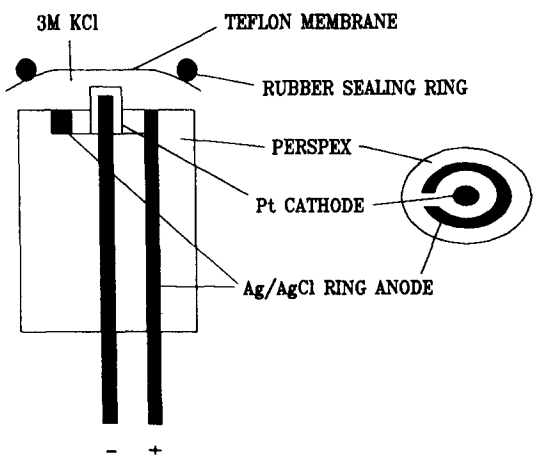

Fig. 7. Design of the Clark-type electrode. An Ag ring anode surrounds a Pt cathode and the circuit is completed by a KCl solution. Such electrodes are usually constructed in Perspex.

The circuit is completed by a KCl-salt bridge between the anode and the cathode and the anode reaction is:

$$Ag + Cl^- \rightarrow AgCl + e^-.$$

Thus, if O_2 is present, a current flows and AgCl is deposited on the anode. As the potential of the cathode is made more negative, O_2 is reduced more rapidly until a potential (difference) is reached where the reduction rate is equal to the rate at which O_2 can diffuse to the cathode, producing a plateau region (diffusion-limited current) (Fig. 8) [73]. If the potential difference is increased beyond the plateau region the evolution of H commences at the cathode and there is a large increase in current. If less O_2 is present its diffusion is slower and the plateau current is, therefore, lower. The amount of current is, thus, linear with the O_2 concentration at the electrode surface.

CO_2 may also be reduced at such a cathode in the following way [74]:
in anhydrous conditions,

$$CO_2 + e^- \rightarrow CO_2^-,$$
$$2CO_2^- \rightarrow CO + CO_3^{2-};$$

in the presence of water,

$$CO_2 + e^- \rightarrow CO_2^-,$$
$$CO_2^- + H_2O \rightarrow HCO_2^- + OH\cdot,$$
$$OH\cdot + CO_2^- \rightarrow HCO_3^-.$$

Hence, an amperometric CO_2 probe is, therefore, possible [75]. This electrode for amperometric CO_2 measurement was a three-electrode system (Fig. 9), consisting of

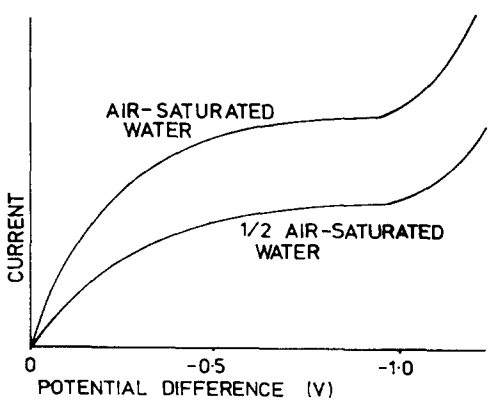

Fig. 8. Current–voltage curve for reduction of O_2 in Clark-type amperometric electrode (Fig. 7). As potential difference between Pt cathode and Ag anode is increased, rate of O_2 reduction at cathode increases. A plateau is reached when reduction rate of O_2 is equal to rate at which O_2 can diffuse to cathode. If potential difference is increased above plateau region, H evolution commences and there is a large increase in current.

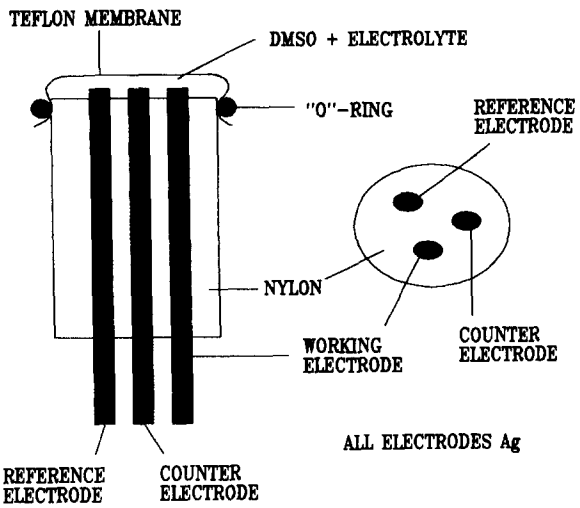

Fig. 9. Design of an amperometric CO_2 electrode [102] based upon the design of an O_2/CO_2 electrode by Albery and Barron [75]. Since Perspex dissolves in DMSO, the electrode was constructed from nylon.

a working electrode, a counter electrode and a reference electrode, as compared to the Clark-type electrode which is generally a two-electrode system (see Fig. 7). In such two-electrode systems, current passing through the reference electrode may cause its potential to deviate and give rise to internal polarisation. This, and resistive drops due to the presence of junction potentials across solutions and the interface between different materials, leads to a decrease in the absolute potential difference between the working electrode and the reference electrode. Addition of the third electrode mimimises these problems as the current now flows between the working electrode and the counter electrode with the potential of the working electrode being set relative to that of the reference electrode [76–78].

To reduce the resistive drops to an insignificant level, inert supporting electrolytes are used [76, 79]. Potassium chloride is commonly used as a supporting electrolyte since it is easily available in high purity form and the mobility of the potassium ion and the chloride ion are almost exactly equal. Potassium nitrate is an alternative when chloride cannot be used. However, when a high concentration of potassium ions is present, the foot of its polarographic reduction wave may extend to values sufficiently positive as to cause unwanted interference [79]. This can be avoided by the use of tetraalkylammonium salts. Interference by the chloride ion often arises when mercury electrodes are used and may be avoided by using nitrate or perchlorate salts.

A number of processes has been reported for the reduction of CO_2 although these have been directed primarily to aqueous solutions [e.g., 80–83]. However, at the potential required for the reduction of CO_2 (at an Ag working electrode the ½-wave potential is -2.13 V vs. the standard calomel electrode [75]) water is electrolysed [84], thus, contra-indicating the use of aqueous solutions within the electrode for analytical purposes. O_2 is much more readily reduced than is CO_2 and, if present, will give rise to the production of superoxide which accumulates and interferes with the measurement

of pCO_2 [75]. In the electrode described by Albery and Barron [75], this problem was circumvented by the use of a metallised membrane as an electrochemical filter for the removal of O_2. The principle of this is that O_2 is reduced, at the metallised membrane in an aqueous solvent to produce water, at a lower potential than that which is required to reduce CO_2. However, in the case of an electrode intended for the monitoring of pCO_2 in anaerobic fermentations the presence of O_2 will not be a problem.

Determination of pCO_2 with an amperometric electrode should be able to give continuous measurements, have a linear relationship between output and pCO_2 (as compared with the Severinghaus-type electrode which has a logarithmic relationship between the variable actually measured, pH and pCO_2 [50]) and be both simple and cheap to construct, especially, if it is to be used under anaerobic conditions.

Recently, a CO_2 sensor using immobilised thermophilic bacteria and an amperometric O_2 electrode was described [85]. The construction of this CO_2 sensor is as shown in Fig. 10. The immobilised bacteria were supplied with O_2-saturated buffer containing various metal ions and 200 μM glucose. CO_2 permeates through the dialysis membrane and is assimilated by the bacteria, causing their respiratory rate to increase [86]. The 'CO_2' concentration was estimated from the decrease in the output current from the O_2 electrode, caused by the decrease in the O_2 concentration corresponding to the increased bacterial respiration. Although the electrode produced an output with a linear relationship to 'CO_2', the range of CO_2 concentrations tested was small, the temperature range was limited and the response time was 5–10 min. CO_2 may be detected by another method which utilises an O_2 electrode [87]. As with the immobilised bacterial sensor, the presence of CO_2 results in a reduction, proportional to the pCO_2, of the current output of the O_2 electrode.

9.3.1. Reduction of CO_2 mediated by an electrocatalyst. Recently, a great deal of attention has been devoted to the utilisation of CO_2 as a source of C in the elec-

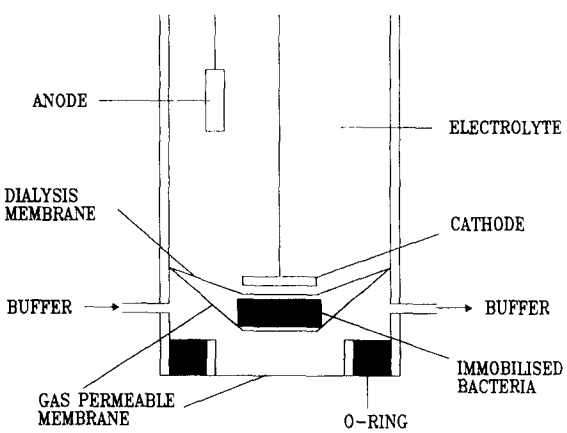

Fig. 10. Cross-section of a CO_2 sensor which utilises immobilised thermophilic bacteria [85]. The bacteria assimilate CO_2 and, in doing so, their respiration rate increases resulting in a decreased O_2 concentration. It is a decrease in O_2 concentration that is measured by the electrode. Such electrodes generally exhibit a rather poor dynamic range.

trochemical synthesis of organic products [e.g., 80, 88]. One possibility would be to use an electrocatalyst, developed for such CO_2 utilisation, in an amperometric CO_2 electrode, to reduce the potential required for the reduction of CO_2. However, few catalytic systems are known despite the development of a number of different strategies (electro-, photo- and photo-electro-chemical). The purely photochemical systems may be classified as either heterogenous, making use of semiconductor suspensions [89–92], or homogenous, employing aqueous solutions of metal ions [82, 93–95], organic dyes or transition metal complexes [96–99]. In a system based on $Re(bipy)(CO)_3Cl$, high yields of CO were photogenerated from CO_2 [100]. When $Re(bipy)(CO)_3Cl$ was used as a catalyst in the electrochemical reduction of CO_2 to CO [83], the system displayed high current efficiency and long-term stability (high overall turnover). Such catalytic properties would appear to make $Re(bipy)(CO)_3Cl$ an attractive catalyst for use in an amperometric CO_2 electrode for the improvement of selectivity and specificity. Direct noncatalysed reduction of CO_2 follows a monoelectronic pathway (equations given above), requiring potentials as negative as -2 V [74, 101]. On the other hand, polyelectronic reduction of CO_2 may occur at much less negative potentials, e.g., the $E_0^{\prime}$ for the dielectronic reduction of CO_2 to CO in aqueous solutions at pH 7 is only -0.52 V [83, 96] and occurs as follows:

$$CO_2 + 2H^+ + 2e^- \leftrightarrow CO + H_2O.$$

$Re(bipy)(CO)_3Cl$ was reported to reduce CO_2 to CO at a potential of -1.25 V, substantially below the potential for monoelectronic CO_2 reduction.

Although the use of $Re(bipy)(CO)_3Cl$ in an electrode may in theory appear to be beneficial, in practice, this electrocatalyst offered no added benefit and, in fact, worsened matters by reducing the rate of current decay after a reduction in pCO_2 [102]. That the use of the electrocatalyst in an electrode caused an increase in the time taken for the current to decrease following a decrease in the pCO_2, was possibly due to interaction between CO_2 and the electrocatalyst. Such interactions would 'delay' CO_2 desorption from the solvent giving rise to higher concentrations of 'CO_2' than would normally be encountered and reflected in the increased current. However, the use of other electrocatalysts in amperometric electrodes may improve this method of CO_2 measurement.

10. Concluding Remarks and Summary

As with any set of analytical methods, there is likely to be a trade-off between resolution, precision, accuracy, response time, ease of use and expense. The methods discussed herein, and summarised in Table 2, cover the spectrum of simplicity. At present, mass spectrometry is probably the most widely used method industrially and/or where money is no object. Fibre optic probes seem to offer many advantages whilst continuing technological development work may be expected for potentiometric devices in particular. IR methods probably suffer too many interferences to be of general use in fermentations though the microwave region does not yet seem to have been explored.

TABLE 2

SUMMARY OF METHODS FOR ESTIMATION OF 'CO_2' DESCRIBED IN TEXT

Method	Applications	Comments
Titrimetric	Liquid samples	Unsuitable for continuous use
Colourimetric	Liquid or gaseous samples	Unsuitable for continuous use
Gravimetric	Exhaust gases	Unsuitable for continuous use
Volumetric	Dissolved 'CO_2'	Not able to provide genuinely continuous monitoring. Cumbersome
Enzymatic	Dissolved 'CO_2'	Specific for 'CO_2' but unsuitable for continuous use
Katharometer	Detection of gases	Not specific for CO_2. Should ideally be combined with a separation method, e.g., GC
Mass spectrometry	Dissolved gases or fermentor exhaust gases	Expensive but precise and accurate
IR	Exhaust gases	Expensive. Not specific for CO_2
Fibre optic probes	Dissolved 'CO_2' Overcomes problems of making miniature glass electrodes	Measures pH – organic acids or bases may interfere
Gas chromatography	Exhaust gas or small liquid samples	Unsuitable for continuous use
Potentiometric	Dissolved 'CO_2'	Slow response time, electrodes not cheap, organic acids/bases may interfere, logarithmic response
Conductiometric	Exhaust gases	Evaluated only up to 0.01 atm
Amperometric	Exhaust gases	Simple and cheap.
Piezoelectric	Exhaust gases	Simple and cheap. Interferences not known but volatile interferences likely

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Noted added in proof

Fatibello-Filho et al. [103] have recently described a CO_2-sensitive piezoelectric crystal which gave a linear response to pCO_2 in the range of 0.018–0.16 atm under fermentation conditions.

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