Formulation of Culture Media for Conductimetric Assays: Theoretical Considerations

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The basic theory of electrolytic conductivity in solutions is described and a model is proposed which allows the direction and relative rates of change of conductivity in microbial cultures to be predicted. Guidelines are presented to enable nutrients to be selected so as to maximize conductivity changes. It is shown that a major consideration in any strategy to maximize conductivity changes in cultures must be to direct as many metabolic activities as possible to act in concert in the production or consumption of protons, and to combine this with use of a pH buffer that exhibits a large change in conductivity on taking up or losing a proton. The ability to predict conductivity changes in microbial systems should permit the rational design of culture media for the selective enumeration of microbes by conductimetric methods and the development of other kinds of conductimetric assays.

INTRODUCTION

From the earliest studies of conductivity changes in microbial systems (Stewart, 1899) it has been hoped that these changes might be related to specific microbial activities, such that conductivity changes might be used to assay these activities. Uschinski (1903) attributed the increase in conductivity in his media to ammonium salts formed by the activities of the bacteria and Oker-Blom (1912) presumed that the initial increase of conductivity in milk cultures was due to conversion of lactose to lactic acid. Parsons & Sturges (1926) obtained excellent correlations between increases in ammonia or ammonia plus amino nitrogen concentrations and increases in the conductivity of cultures of clostridia in various proteinaceous media. With further developments in the understanding of bacterial metabolism, Allison et al. (1938) could write . . . some kind of a relationship between variables such as NH₃, CO₂, [H⁺], numbers of bacteria, specific conductivity etc. must exist . . . These speculations emphasize the fact that if the mechanisms of coordination between these variables were known, then the measurement of a single variable such as specific conductivity would permit an integration of the whole process of metabolism.'

The current interest in and commercial application of conductimetric and impedimetric techniques to the assessment of microbial populations in medical, pharmaceutical and food samples dates from papers by Cady (1975), Ur & Brown (1975) and Richards et al. (1978). Although Richards et al. (1978) gave a lucid description of the basic nature of the processes involved in conductivity changes in cultures, there has been essentially no progress towards understanding the relationships between metabolic activities and conductivity changes since the statements of Allison et al. (1938). So poor, in fact, is the theoretical knowledge that it is not always possible to anticipate whether a particular organism–medium combination will lead to an increase or a decrease in conductivity of the medium. Eden & Eden (1984) and Firstenberg-Eden & Eden (1984) described an 'electro-bacteriological' model but this embodies only the general principle that the conductivity change is proportional to the amount of growth and does not help in the design of culture media for conductimetric assays.

Consequently, the application of conductimetric and impedimetric methods to the assay of microbial populations has been almost entirely dependent on the empirical development of
suitable culture media. Such an approach with systems as complex as microbe–culture medium interactions is expensive and yields data having limited predictive value. Methodologies whose underlying basic mechanisms are unknown may be inherently unreliable. For instance, McMurdo & Whyard (1984) described the case of a heat-treated skim milk powder that was heavily contaminated with coryneform bacteria but which gave a constant impedimetric response irrespective of the number of coryneform bacteria present. It appeared that the coryneform bacteria were outgrown by the small number of Bacillus in the powder, and the impedimetric response recorded was thus effectively a function of the number of Bacillus present. The importance to the interpretation of biochemical conductimetric assays of understanding all the sources of conductivity changes in microbial systems has recently been emphasized by Owens et al. (1985).

It is evident that wider exploitation of conductimetric techniques in microbiology and biochemistry requires a thorough understanding of the sources of conductivity changes. Once these are known media may be rationally formulated so as to maximize conductivity changes and hence enhance their sensitivity for the enumeration of microbes. Additionally, it may become easier to design media for the enumeration and/or identification of specific microorganisms or metabolic activities. This paper describes a model that provides such a framework. The simple theory of conductivity in solutions and the factors affecting it are first described. The available simple theoretical treatments are strictly applicable only to dilute solutions containing a small number of different kinds of ions. Nevertheless, their application to microbial cultures does allow many of the factors that determine the direction and rate of conductivity changes in cultures to be identified. Such information is quite adequate for the rational formulation of culture media for conductimetric assays. Changes in impedance (the resistance to the flow of an alternating current, comprising a conductance and a capacitance component) in microbial cultures are even less well understood than conductivity changes (Firstenberg-Eden & Eden, 1984). Since the exact mechanisms by which microbial growth might affect capacitance are unknown, impedance is not considered in this paper.

**BASIC THEORY OF ELECTRICAL CONDUCTIVITY IN SOLUTIONS**

The following account is largely compiled from Bockris & Reddy (1970), Crow (1979) and Davies (1967).

When two electrodes are placed in an electrolyte-containing solution and a potential difference is applied across them a drift of ions is produced in which ions move towards the electrode of opposite charge. The steady flow of charge (current) is maintained by ions taking electrons from or handing electrons to the electrodes. The ability of a solution to conduct current is evaluated from measurement of the resistance of the solution. The current flowing through a conductor under the influence of a constant e.m.f. is inversely proportional to its resistance, \( R \) (expressed in ohms, \( \Omega \)). Hence, \( 1/R \) is a measure of the conducting power and is called the conductance, \( G \), of the solution (expressed in reciprocal ohms or siemens, S).

The resistance of any conductor is given by:

\[
R = \rho (l/A)
\]

where \( l \) is the length of the conductor (cm), \( A \) is the cross-sectional area of the conductor (cm\(^2\)), and \( \rho \) is the specific resistance or resistivity of the material (\( \Omega \) cm). Resistivity is a property of the material and its reciprocal is called the conductivity, \( \kappa \), of the material:

\[
\kappa = 1/\rho = (1/R)(l/A) \quad (\Omega^{-1} \text{ cm}^{-1} \text{ or } S \text{ cm}^{-1})
\]

Hence, the electrolytic conductivity of a solution is equal to the conductance of a portion 1 cm in length and 1 cm\(^2\) in cross-section.

To evaluate the conductivity of a solution, the resistance is measured of a portion of the solution between electrodes of fixed area and held at a fixed separation from each other. Because of the difficulty of accurately measuring the area and separation of electrodes, it is usual to first determine a cell constant \( (l/A) \) by measuring the resistance of a solution of accurately known
conductivity in the cell. The electrolytic conductivity of any electrolyte-containing solution is then given by its measured conductance between these electrodes multiplied by the cell constant:

$$\kappa = (1/R)(l/A)$$

Since current in solutions is carried only by ions, it is evident that conductivity will vary with the concentration, $C$, of electrolyte:

$$\kappa = f(C)$$

Hence, in order to compare the conducting power of different electrolytes it is necessary to define a parameter for a standard quantity of electrolyte. The molar conductivity, $\Lambda$, of an electrolyte may be defined as the conductivity of a solution (i.e. the conductance of a portion 1 cm$^2$ by 1 cm) divided by the concentration of electrolyte ($C$, mol cm$^{-3}$):

$$\Lambda = \kappa/C \text{ (S cm}^2\text{ mol}^{-1})$$

Thus molar conductivity is a measure of the conducting power of 1 mole of electrolyte at any concentration, and the conductivity of a solution at a particular concentration, $c$ (mol l$^{-1}$), is given by:

$$1000\kappa = Ac \text{ (S cm}^{-1})$$

where the 1000 multiplier converts concentrations to mol l$^{-1}$. Molar conductivity is defined as above, rather than as the conductivity at a fixed concentration, because it is not a constant parameter but varies with concentration. As a solution of an electrolyte is made more dilute the molar conductivity increases and approaches a limiting value called the molar conductivity at infinite dilution, $\Lambda_0$. This change is attributed to the lessening of interionic interactions as the ions become further apart with dilution.

**Quantitative relationships between molar conductivity and electrolyte concentration**

For strong electrolytes Kohlraush established the empirical relationship:

$$\Lambda = \Lambda_0 - Kc^{1/2}$$

where $K$ is a constant mainly controlled by the valency of the ions. For uni-univalent salts (e.g. KCl, NaCl) this equation holds up to concentrations of about 0.005 mol l$^{-1}$. Extrapolation of the plot of $\Lambda$ against $c^{1/2}$ allows determination of $\Lambda_0$. Onsager used the interionic attraction theory of Debye and Hückel to provide a theoretical basis for the Kohlraush relationship:

$$\Lambda = \Lambda_0 - (a\Lambda_0 + b)c^{1/2}$$

where $a$ and $b$ are constants depending upon the valencies of the two ions, the dielectric constant of the solvent, its viscosity, and the absolute temperature. For uni-univalent salts in water at 25 °C, $a = 0.2292$ and $b = 60.22$.

The Onsager equation is quite successful at predicting the initial fall in conductivity with increase in concentration for simple solutions of uni-univalent salts up to concentrations of about 0.001 mol l$^{-1}$ and of some uni-divalent salts up to rather lower concentrations, but no case is known in which a salt with a valency product of 4 or more obeys the equation at accessible concentrations. This is believed to be due to the occurrence in these solutions of ion-pairs held together by the strong electrostatic forces to which multivalent ions are subject. As a result of these forces, a pair of oppositely charged ions coming into close contact remain for a time under each other’s influence and lose their independent identities. Thus a solution of Na$_2$SO$_4$ is envisaged as including the species NaSO$_4^-$ as well as Na$^+$ and SO$_4^{2-}$ and its conductivity is therefore lower than predicted by the Onsager equation. For example, whereas the molar conductivity of 0.01 m-KCl is approximately 94% of the value at infinite dilution, that of 0.01 m-Na$_2$SO$_4$ is 87% and that of 0.01 m-CuSO$_4$ is 62%. At concentrations of 0.1 m the figures are 86, 69 and 38% of the respective molar conductivities at infinite dilution (Conway, 1952).
Equations have been developed that take account of ion-pair formation but they also are only successful at concentrations substantially lower than those relevant to microbiological culture media (e.g., Pethybridge, 1982) and their further consideration here is not justified.

**Effect of total ionic strength on conductivity**

All the ions in a solution will contribute to the ion atmospheres and thus it is the total ionic concentration that governs the decrease in conductivity from that at infinite dilution. The ion atmosphere effects are determined by the ionic strength $I$:

$$I = 0.5 \sum z_i^2 c_i$$

where $z$ is the numerical value of the charge of the $i$th ion, and $c$ is its concentration (mol l$^{-1}$). For example, in a solution which is 0.001 m in NaCl and 0.001 m in KCl, the ionic strength is 0.002 mol l$^{-1}$ and, as a first approximation, the conductivity of the solution may be calculated using the molar conductivities at $c$ equal to 0.002 mol l$^{-1}$.

$$1000 \kappa = (\Lambda_{\text{NaCl}}^{0.002} \times 0.001) + (\Lambda_{\text{KCl}}^{0.002} \times 0.001)$$

where $\Lambda_{\text{NaCl}}^{0.002}$ is the molar conductivity of 0.002 m-NaCl, and $\Lambda_{\text{KCl}}^{0.002}$ is the molar conductivity of 0.002 m-KCl.

Due to the charge-squared term in the calculation of $I$, bivalent or higher valent ions have a greater effect on ionic strength than univalent ions and hence their presence in solutions results in a greater depression of the conductivity than the equivalent amount of univalent ions.

**Effect of temperature on conductivity**

Molar conductivity increases with increase in temperature due, primarily, to the decrease in the viscosity of the solvent with increasing temperature. For nearly all ions except $\text{H}^+$ and $\text{OH}^-$ the rate of change is $1.018 - 1.022 \degree \text{C}^{-1}$. For $\text{H}^+$ it is $1.042 \degree \text{C}^{-1}$ and for $\text{OH}^-$ it is $1.016 \degree \text{C}^{-1}$. Richards et al. (1978) reported a mean value of $1.016 \degree \text{C}^{-1}$ for some common culture media. Hence, when conductivities, and especially changes in conductivities, are determined it is very important that temperature control is precise. In the absence of compensation for temperature changes, to detect changes in conductivity with a precision of 1 in $10^5$, temperature must vary by less than 0.0006 $\degree \text{C}$ (Richards et al., 1978).

**Effects of other factors on conductivity**

Two other factors that can substantially influence the conductivity of solutions are the viscosity and the dielectric constant of the solvent. However, in most liquid aqueous culture media neither of these is likely to have values greatly different from those of pure water.

**Conductivity of mixtures of electrolytes**

In principle the conductivity of mixtures of electrolytes may be calculated by summing the conductivities of the individual ions. For example, for a mixture of 0.001 m-KCl and 0.001 m-NaCl:

$$1000 \kappa = (\lambda_{\text{Na}} \times 0.001) + (\lambda_{\text{K}} \times 0.001) + (\lambda_{\text{Cl}} \times 0.002)$$

where $\lambda$ is the molar conductivity of the respective ions (S cm$^2$ mol$^{-1}$). However, as indicated above, while it is possible to calculate the molar conductivities of ions in dilute solutions containing two or three ion types with reasonable accuracy, it is not feasible to calculate accurate values in more complex solutions even when dilute, let alone at the concentrations existing in culture media.

In solutions of complex mixtures of uni- and higher valent ions, ion-pair formation is likely to be very significant. For example, in seawater it is estimated (Butler, 1982) that sulphate is present as (approximate % of total): $\text{SO}_4^{2-}$, 50; $\text{MgSO}_4$ ion-pairs, 25; $\text{NaSO}_4$, 20; $\text{CaSO}_4$, 4; $\text{KSO}_4$, 1; carbonate is present as $\text{CO}_3^{2-}$, 10; $\text{MgCO}_3$, 67; $\text{CaCO}_3$, 7; $\text{NaCO}_3$, 17; and bicarbonate is present as $\text{HCO}_3^-$, 71; $\text{MgHCO}_3$, 17; $\text{NaHCO}_3$, 8; $\text{CaHCO}_3$, 3. The percentages of metal ions present as free cations are estimated to be: $\text{Na}^+$, 99; $\text{K}^+$, 98; $\text{Mg}^{2+}$, 86 and $\text{Ca}^{2+}$, 88.
Culture media for conductimetric assays

(i.e. the major anion is Cl\(^-\) which is not involved in ion-pairing). Thus ion-pairing obviously removes many ions from current carrying and will have a large effect on the conductivity of the mixture. It also results in a lowering of the total ionic strength, from 0.71 to 0.66 mol kg\(^{-1}\) in the case of seawater, and acid–base equilibria are affected by the removal of ions, such as HCO\(_3\)^- and CO\(_3\)^{2-}, from their acid–base equilibria.

CONDUCTIVITY CHANGES IN MICROBIAL CULTURES

Microbial culture media contain a great diversity of ionic species and it can be anticipated that substantial ion-pairing and other kinds of interionic interactions will occur, such that the calculation of absolute conductivities of culture media is not feasible. However, since we are concerned only with identifying the sources of the conductivity changes that occur in cultures as a result of the metabolic activities of micro-organisms, it is not essential that conductivity calculations yield accurate values for them to be useful. Rather, for the purpose of the rational formulation of culture media for conductimetric assays, it will suffice if they allow the relative rates of change and their directions to be predicted for different medium–micro-organism combinations.

A micro-organism may be represented as a compartment engaged in exchanging compounds with the external environment. These compounds may be classed as electron donors, final electron acceptors, carbon sources, nitrogen sources, other inorganic nutrients and metabolic products, all of which may be charged or uncharged (Fig. 1). Although microbial cells often carry a net negative charge it will be assumed here that cells are electrically neutral and not involved in carrying current. Even though this is not strictly true, the conductivity of microbial cells is negligible compared with the changes in conductivity of small ions associated with growth (Richards et al., 1978). It is also assumed that any net charge accumulated by microbial cells is negligible compared with the overall charge flux. Hence, the uptake of charged ions must be balanced by uptake of oppositely charged ions or excretion of similarly charged ones. In aqueous culture media any net uptake or excretion of charge can be presumed to be balanced by uptake or excretion of H\(^+\) or OH\(^-\) ions (Roos & Luckner, 1984). If these react with pH buffer compounds in the medium then this is liable to lead to changes in the conductivities of the buffers. One other possible source of conductivity changes in cultures shown in Fig. 1 is the hydrolysis of polymers by excreted exoenzymes.

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**Fig. 1.** Diagrammatic representation of a microbial cell and its interactions with the external environment. E\(^{+0}\), R\(^{+0}\), etc. represent compounds with net positive, negative or zero charge.
Calculation of conductivity changes in cultures

In principle, the conductivity change in a culture medium as a consequence of microbial growth can be calculated by summing the net effects of all the processes illustrated in Fig. 1. To do this requires that a number of assumptions be made.

(i) Removal of ions from current carrying due to ion-pair formation or chelation is neglected.
(ii) Current carried by microbial cells or charged polymers such as proteins is neglected.
(iii) Any net accumulation of charge by microbial cells is neglected.
(iv) There is only one compound that functions as a pH buffer at the pH of the culture. If the culture produces CO₂ then buffering due to CO₂/HCO₃ may also need to be taken into account. None of the other electron donors, electron acceptors, carbon sources, nitrogen sources or metabolic products function as pH buffers in the pH range of the culture.
(v) Conductivity due to H⁺ and OH⁻ is neglected. In the pH range of about 3–10 the concentrations of these ions are so low compared with those of other ionic species that this assumption is justified.
(vi) The effects of ionic strength, and changes in ionic strength, on pKₐ values are neglected (if desired, these effects could be allowed for relatively easily).
(vii) The amount of any exoenzymes excreted is proportional to the amount of growth and, once synthesized, exoenzymes are not degraded.
(viii) The viscosity of the culture medium does not change sufficiently during growth to have a significant effect on the molar conductivities of the ions present.

Total conductivity of culture medium

Accepting the above assumptions, the conductivity of a culture medium at any instant is then given by the sum of the product of the concentration of each ionic species and its molar conductivity:

\[ 1000 \kappa_T = [E^+]\lambda_E + [R^+]\lambda_R + [C^+]\lambda_C + [N^+]\lambda_N + [M^+]\lambda_M + [A^-]\lambda_A + [P^+]\lambda_P + [B^+]\lambda_B + [BH^+]\lambda_{BH} + [P^-]\lambda_{P^-} \]

where \( \kappa_T \) is the total conductivity of the culture medium (S cm⁻¹), \([E^+], [R^+], \text{etc.} \) are the concentrations of ions bearing one or more net positive or negative charges (mol l⁻¹) and letters within the brackets have the same meanings as in Fig. 1, and \( \lambda \) is the molar conductivity (S cm² mol⁻¹) of the ion indicated by the subscript.

Change in conductivity with growth

The change in conductivity of the medium for a given amount of growth is given by:

\[ \Delta \kappa_T = \Delta \kappa_E + \Delta \kappa_R + \Delta \kappa_C + \Delta \kappa_N + \Delta \kappa_M + \Delta \kappa_P + \Delta \kappa_{BH} + \Delta \kappa_{P^-} \]

where \( \Delta \kappa_E, \Delta \kappa_R, \text{etc.} \) are the changes in the conductivity of the component of the medium indicated by the subscript.

Change in conductivity due to electron donor, electron acceptor, carbon source, nitrogen source and other inorganic nutrients. These are given by the following relationships, where subscript 1 indicates a final state after growth has occurred:

\[ 1000 \Delta \kappa_E = -(X_1/Y_E)\lambda_E \quad 1000 \Delta \kappa_R = -(X_1/Y_R)\lambda_R \]
\[ 1000 \Delta \kappa_C = -(X_1/Y_C)\lambda_C \quad 1000 \Delta \kappa_N = -(X_1/Y_N)\lambda_N \]
\[ 1000 \Delta \kappa_M = -\Sigma(X_1/Y_\text{MA})\lambda_{MA} \]

where \( X_1 \) is the concentration of biomass formed (g dry biomass l⁻¹), \( Y \) is the yield coefficient for the nutrient indicated by the subscript (g dry biomass mol⁻¹), and MA refers to any inorganic cation or anion.

Change in conductivity due to excreted products other than CO₂. \( 1000 \Delta \kappa_P = \Sigma(nX_1/Y)\lambda_P, \) where \( Y \) is the yield coefficient with respect to the nutrient precursor of the product (i.e. \( Y_E, Y_R, Y_C \) or \( Y_N, \) g dry biomass mol⁻¹), and \( n \) is the number of moles of product formed per mole of precursor nutrient consumed.
Change in conductivity due to the pH buffer. The net concentration of H⁺ leaving the cell for a given concentration of biomass produced is given by:

\[ H^+_{\text{out}} = \Sigma z_i \Delta [I^+]_{\text{in}} - \Sigma z_i \Delta [I^-]_{\text{in}} + \Sigma z_i \Delta [P^-]_{\text{out}} - \Sigma z_i \Delta [P^+]_{\text{out}} \]

where \( H^+_{\text{out}} \) is the net concentration of H⁺ excreted (mol l⁻¹), \( \Delta [I^+]_{\text{in}} \) is the change in concentration of any ion bearing positive charge, \( z_i \), taken up by cells (i.e. \( E^+, R^+, C^+, N^+, M^+ \), mol l⁻¹), \( \Delta [I^-]_{\text{in}} \) is the change in concentration of any ion bearing negative charge, \( z_i \), taken up by cells (i.e. \( E^-, R^-, C^-, N^-, A^- \), mol l⁻¹), \( \Delta [P^-]_{\text{out}} \) is the change in concentration of any ion bearing negative charge, \( z_i \), excreted by cells (mol l⁻¹), and \( \Delta [P^+]_{\text{out}} \) is the change in concentration of any ion bearing positive charge, \( z_i \), excreted by cells (mol l⁻¹).

In the buffering zone of the pH buffer (i.e. \( \text{pH} \sim pK_a \pm 1 \)) the \( H^+_{\text{out}} \) will be entirely absorbed by the buffer (since any change in \([H^+]\) is negligible compared with \( H^+_{\text{out}} \)):

\[ B^\pm + H^+_{\text{out}} \rightarrow BH^\pm \]

where \( B^\pm \) represents the conjugate base bearing net positive, negative or zero charge, and \( BH^\pm \) represents the conjugate acid bearing net positive, negative or zero charge. Thus:

\[ 1000\Delta \kappa_{BH/BH^\pm} = (H^+_{\text{out}})(\lambda_{BH} - \lambda_B) \]

where \( \lambda_{BH} \) is the molar conductivity of conjugate acid and \( \lambda_B \) is the molar conductivity of conjugate base.

Correction to calculations for pH buffer and excreted products to take account of CO₂ production. Since \( \text{CO}_2 \) is such a frequent product of microbial metabolism, and has a \( pK_a \) of 6.35, it is necessary (if the \( \text{CO}_2 \) is retained in the culture) to correct the calculations for \( H^+_{\text{out}} \) and those for changes in conductivity to take account of the proportion of \( \text{CO}_2 \) present as \( \text{HCO}_3^- \). The effect of the hydrolysis of \( \text{CO}_2 \) to bicarbonate is the formation of an additional \( \text{H}^+ \) and the conversion of an additional molecule of buffer conjugate base to conjugate acid:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+ \]

Adding:

\[ \text{CO}_2 + \text{H}_2\text{O} + B^\pm \rightarrow \text{HCO}_3^- + BH^\pm \]

The extent to which this reaction occurs depends upon the \( pK_a \) of the particular buffer, and may be calculated as follows.

Since

\[ [H^+]_i = \frac{K^\prime_i(C^C_T - [\text{HCO}_3^-])_i}{[\text{HCO}_3^-]_i} \]

and

\[ [H^+]_i = \frac{K^B[HB]_i}{[B]_i} = \frac{K^B([HB]_i + [\text{HCO}_3^-])_i)}{[B]_i - [\text{HCO}_3^-]_i} \]

where \( K^\prime_i \) is the first dissociation constant for carbonic acid, \( C^C_T \) is the total concentration of \( \text{CO}_2 \) produced (mol l⁻¹), \( [\text{HCO}_3^-]_i \) is the final concentration of \( \text{HCO}_3^- \) (mol l⁻¹), \( K^B \) is the dissociation constant for the pH buffer, \( [B]_i \) is the final concentration of the conjugate base of the buffer (mol l⁻¹), \( [BH]_i \) is the final concentration of the conjugate acid of the buffer (mol l⁻¹), \( [B]_i \) is the ‘transition’ concentration of B calculated without taking \( \text{HCO}_3^- \) formation into account, and \( [BH]_i \) is the ‘transition’ concentration of HB calculated without taking \( \text{HCO}_3^- \) formation into account. Thus:

\[ \frac{K^\prime_i(C^C_T - [\text{HCO}_3^-])_i}{[\text{HCO}_3^-]_i} = \frac{K^B([BH]_i + [\text{HCO}_3^-])_i)}{[B]_i - [\text{HCO}_3^-]_i} \]
Rearranging:

\[(K_C^T - K_B)([\text{HCO}_3^-])_1^2 - (K_B^T[B]_1 + K_C^T C_T^T + K_C^T[B]_1)\text{[HCO}_3^-])_1 + K_C^T C_T^T[B]_1 = 0\]

Thus \([\text{HCO}_3^-])_1\) can be calculated by solving this quadratic equation and the final pH determined from:

\[[\text{H}^+]_1 = \frac{K_B^T([\text{HB}]_1 + [\text{HCO}_3^-])_1)}{[\text{B}]_1 - [\text{HCO}_3^-])_1}\]

The concentrations of B and BH can then be determined, since:

\[K_B = \frac{[\text{B}]_1[\text{H}^+]_1}{[\text{BH}]_1} = \frac{[\text{B}]_1[\text{H}^+]_1}{C_T - [\text{B}]_1}\]

thus

\[[\text{B}]_1 = \frac{C_T^T K_B}{[\text{H}^+]_1 + K_B}\]

and

\[[\text{BH}]_1 = C_T^T - [\text{B}]_1\]

where \(C_T^T\) is the total concentration of buffer present.

Change in conductivity due to hydrolysis of polymers external to the cells. Since it is assumed that the concentration of exoenzyme produced is proportional to the amount of growth, the amount of hydrolysis of external polymers in a culture growing at a constant rate under constant conditions will also be proportional to the concentration of biomass formed:

\[[\text{P}_r]_1 = X_1 Y_{pr}\]

where \(X_1\) is the concentration of biomass formed (g dry biomass \(1^{-1}\)) and \(Y_{pr}\) is the yield coefficient for polymer hydrolysis [mol products (g biomass)\(^{-1}\)]. Thus:

\[1000\Delta\kappa_{pr} = X_1 Y_{pr} \lambda_{pr}\]

where \(\Delta\kappa_{pr}\) is the change in conductivity due to polymer hydrolysis, and \(\lambda_{pr}\) is the molar conductivity of the product of the hydrolysis.

Total change in conductivity of culture media due to microbial growth. Using the arguments presented above, it is possible to determine the changes in the concentrations of all the ionic species present following a specified amount of microbial growth, but what are not known are the molar conductivities of the ions under the prevailing conditions. As indicated earlier, it is not possible to calculate accurately the molar conductivities of ions in complex mixtures. Hence it is necessary to assume, as a first approximation, that the molar conductivities of the different ions are reduced proportionately by similar amounts from their values at infinite dilution. (This assumption is not strictly true even for dilute solutions of 1:1 electrolytes since the reduction is given by \((a\Lambda_0 + b)c^{1/2}\) in the Onsager equation. In mixtures of electrolytes involving significant ion-pairing, it certainly involves some degree of error. If greater precision were required the extent of ion-pair formation could be roughly estimated, but this is not justified here.) Thus, for the purposes of comparing the relative contributions of the changes in the conductivity of each of the medium components to the total change in conductivity of the medium, molar conductivities at infinite dilution may be used. The molar conductivities at infinite dilution of some ions relevant to microbial cultures are listed in Table 1.

In Table 2 the calculated relative contributions of different medium components are shown for a hypothetical organism growing on glucose by a homolactic fermentation. It is apparent that the overall change in conductivity is a consequence of decreases in some components of the medium and increases in others. It follows that the overall change in medium conductivity could be maximized by ensuring that the changes in the conductivity of the components exhibiting the larger changes are all in the same direction.
Table 1. *Molar conductivities at infinite dilution of ions in aqueous solution at 25 °C*

The values are taken from: a, Stark & Wallace (1982); b, Erdely-Gruz (1974); c, Parsons (1959); d, Pethybridge & Taba (1982); e, Pethybridge et al. (1983).

<table>
<thead>
<tr>
<th>Cation</th>
<th>( \lambda_0 ) (S cm(^2) mol(^{-1}))</th>
<th>Anion</th>
<th>( \lambda_0 ) (S cm(^2) mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(^+)</td>
<td>349.8(^a)</td>
<td>OH(^-)</td>
<td>198.6(^a)</td>
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<td>K(^+)</td>
<td>73.5(^a)</td>
<td>Cl(^-)</td>
<td>76.4(^a)</td>
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<td>50.1(^a)</td>
<td>NO(_3^-)</td>
<td>71.4(^a)</td>
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<tr>
<td>NH(_4^+)</td>
<td>73.5(^a)</td>
<td>S(_2O_3^2^-)</td>
<td>152.0(^d)</td>
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<tr>
<td>Mg(^{2+})</td>
<td>106.1(^d)</td>
<td>S(_2O_3^2^-)</td>
<td>174.4(^d)</td>
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<tr>
<td>Ca(^{2+})</td>
<td>118.0(^d)</td>
<td>SO(_2^-)</td>
<td>159.6(^d)</td>
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<td>H(_2)PO(_4^2^-)</td>
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<tr>
<td>HOC(_2)H(_2)NH(_3^+)</td>
<td>42.2(^c)</td>
<td>HPO(_4^2^-)</td>
<td>114(^b)</td>
</tr>
<tr>
<td>C(_6)H(_5)NH(_3^+)</td>
<td>41(^c)</td>
<td>PO(_4^3^-)</td>
<td>240(^b)</td>
</tr>
<tr>
<td>(C(_6)H(_5))(_2)NH(_2^+)</td>
<td>29(^c)</td>
<td>HCO(_3^-)</td>
<td>44.5(^b)</td>
</tr>
<tr>
<td>(CH(_3))(_2)NH(_3^+)</td>
<td>42(^c)</td>
<td>CO(_3^-)</td>
<td>118.6(^d)</td>
</tr>
<tr>
<td>(C(_2)H(_6))(_2)NH(_2^+)</td>
<td>34(^c)</td>
<td>HCOO(^-)</td>
<td>54.6(^d)</td>
</tr>
<tr>
<td>(CH(_3))(_2)N(^+)</td>
<td>44.9(^d)</td>
<td>CH(_3)COO(^-)</td>
<td>40.9(^d)</td>
</tr>
<tr>
<td>(C(_2)H(_6))(_2)N(^+)</td>
<td>32.7(^c)</td>
<td>HOOC(_3ICOO^-)</td>
<td>40.2(^c)</td>
</tr>
<tr>
<td>(C(_3)H(_3))(_2)N(^+)</td>
<td>23.5(^e)</td>
<td>C(_2)H(_5)COO(^-)</td>
<td>35.8(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C(_2)H(_5)COO(^-)</td>
<td>35.1(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C(_2)H(_4)COO(^-)</td>
<td>33.4(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH(_2)CHCHCHCHCOO(^-)</td>
<td>31.3(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(COO(^-))(_2)</td>
<td>148.4(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tr-(CHCOO(^-))(_2)</td>
<td>123.6(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(CH(_2)COO(^-))(_2)</td>
<td>114.6(^d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(CH(_3))(_3)(COO(^-))(_2)</td>
<td>105.2(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(CHOHCOO(^-))(_2)</td>
<td>119.2(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH(_3)CHOH(COO(^-))(_2)</td>
<td>117.6(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C(_6)H(_4)COO(^-)</td>
<td>32.3(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o-C(_6)H(_4)(COO(^-))(_2)</td>
<td>104.6(^c)</td>
</tr>
</tbody>
</table>

Table 2. *Calculation of conductivity changes due to growth of a hypothetical bacterium on glucose by homolactic fermentation in the presence of phosphate buffer*

<table>
<thead>
<tr>
<th>Metabolic activity</th>
<th>Metabolite flux* [mmol (g dry biomass)(^{-1})]</th>
<th>H(^+) flux [mmol H(^+) out (g dry biomass)(^{-1})]</th>
<th>Change in conductivity [mS cm(^{-1})]</th>
<th>Percentage of net change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron donor</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbon source</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nitrogen (NH(_4^+))</td>
<td>8</td>
<td>+8</td>
<td>-0.59</td>
<td>+15</td>
</tr>
<tr>
<td>Other nutrients: K(^+)</td>
<td>0.43</td>
<td>+0.65</td>
<td>-0.12</td>
<td>+3</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.008</td>
<td>-1.45(^d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H(_2)PO(_4^2^-)/HPO(_4^2^-)</td>
<td>0.83</td>
<td>+66</td>
<td>+3.28(^d)</td>
<td>-59</td>
</tr>
<tr>
<td>SO(_4^2^-)</td>
<td>0.10</td>
<td>+66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product (lactate)</td>
<td>66</td>
<td>+66</td>
<td>+2.38(^d)</td>
<td>-5.71</td>
</tr>
<tr>
<td>Buffer (HPO(_4^2^-) (\rightarrow) H(_2)PO(_4^2^-))</td>
<td>73.20</td>
<td>+66</td>
<td></td>
<td>+141</td>
</tr>
<tr>
<td>Totals</td>
<td>+73.20</td>
<td>+66</td>
<td>-4.04</td>
<td>100</td>
</tr>
</tbody>
</table>

* Yield coefficients based on Pirt (1975).
† Calculated using molar conductivities at infinite dilution.
‡ Assuming that equal amounts of H\(_2\)PO\(_4^2^-\) and HPO\(_4^2^-\) are assimilated.
§ Calculated using an estimated molar conductivity at infinite dilution for lactate of 36 S cm\(^{-1}\) mol\(^{-1}\).
Table 3. Calculated relative conductivity changes in microbial cultures in different culture media to illustrate the effect of carbon and energy source, nitrogen source, and electron acceptor

The values were calculated using the following assumptions: yield coefficients based on Pirt (1975); carbon content of cell = 0.5 g (g dry biomass)^{-1}; pH changes occur within the buffering range of the phosphate buffer; conductivity changes calculated using molar conductivities at infinite dilution without correction for ionic strength; the energy-yielding metabolism shown applies to that portion of the carbon and energy source not assimilated.

<table>
<thead>
<tr>
<th>Energy-yielding metabolism</th>
<th>Yield [g dry biomass (mol C-source)^{-1}]</th>
<th>Nitrogen source</th>
<th>Conductivity change [μS cm^{-1} (100 mg dry biomass l^{-1})^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_6H_{12}O_6 + 6O_2 → 6CO_2 + 6H_2O</td>
<td>80</td>
<td>NH_4^+</td>
<td>-130</td>
</tr>
<tr>
<td>C_6H_{12}O_6 + H_2O → lactate + 0.5 acetate + 0.5 ethanol + 0.5 HCO_3^- + 0.5 CO_2 + H_2 + 2H^+</td>
<td>27</td>
<td>NH_4^+</td>
<td>-360*</td>
</tr>
<tr>
<td>C_6H_{12}O_6 + (CH_3)_3NO + 0.5H_2O → lactate + acetate + (CH_3)_3NH^+ + 0.5 HCO_3^- + 0.5 CO_2 + H_2 + 1.5H^+</td>
<td>30</td>
<td>NH_4^+</td>
<td>-60*</td>
</tr>
<tr>
<td>C_6H_{12}O_6 + 3SO_2^- + 1.5H_2O → lactate + acetate + 2SO_4^{2-} + 0.5 HCO_3^- + 0.5 CO_2 + H_2 + 4.5H^+</td>
<td>30</td>
<td>NH_4^+</td>
<td>-280*</td>
</tr>
<tr>
<td>(CH_2COO)^{-2} + 3.5O_2 + 2H^+ → 4CO_2 + 3H_2O</td>
<td>40</td>
<td>NH_4^+</td>
<td>-25</td>
</tr>
<tr>
<td>as above</td>
<td>40</td>
<td>NO_3^-</td>
<td>+100</td>
</tr>
<tr>
<td>C_3H_7NH_3^+ + 4.5O_2 → 3CO_2 + 3H_2O + NH_4^+</td>
<td>25</td>
<td>NH_4^+</td>
<td>+5</td>
</tr>
<tr>
<td>CH_2CHNH_2COO^- + 3O_2 + H^+ → 3CO_2 + 2H_2O + NH_4^+</td>
<td>30</td>
<td>NH_4^+</td>
<td>+370</td>
</tr>
</tbody>
</table>

* Estimated, taking λ_0 for lactate as 36 S cm^2 mol^{-1}.

It is also evident that, for this hypothetical culture, the medium component showing the greatest quantitative change in conductivity is the phosphate pH buffer. This is an inevitable consequence of the large H^+ flux. Thus a major consideration in any strategy to maximize conductivity changes in microbial cultures must be to direct as many metabolic processes as possible to act in concert in the production/consumption of H^+, and to combine this with the use of a pH buffer compound that exhibits a large change in conductivity on taking up or losing a proton.

The calculations presented in Table 2 are relatively simple, but they become much more complicated when more reactions, and CO_2 effects, are introduced. Table 3 shows the results of some calculations, made using a computer simulation program, that illustrate the effects of different medium compositions and microbial activities on the predicted conductivity changes in the cultures.

**Approaches to the maximization of conductivity changes in culture media**

The model presented here leads to a number of principles that can be applied to the formulation of culture media to maximize the conductivity changes that occur with microbial growth. The main factors to be considered, in approximate order of importance, are: (i) selection of pH buffer, (ii) H^+ flux, (iii) electron donor, (iv) external electron acceptor, (v) carbon source, (vi) nitrogen source, (vii) products of metabolism, (viii) other inorganic nutrients, (ix) other metabolic activities, (x) general considerations. These will be considered in turn.

**Selection of pH buffer**

The pH buffer selected should exhibit a large change in molar conductivity (ΔΛ_0, at infinite dilution) upon gaining or losing a proton, so that it amplifies the H^+ flux due to metabolic activities. In addition, the direction of the change should be such as to aid other conductivity changes in the medium.
Culture media for conductimetric assays

The buffer compounds should also possess the desirable features described by Good & Izawa (1972). In particular, compounds that are charged in both their acid and their basic forms are to be preferred over uncharged compounds since there is less risk of toxic effects. Since the buffering range is approximately $pK_a + 1$, in order to have buffers suitable for both bacterial and fungal cultures, it is desirable, at least, to have available compounds with the following properties:

(a) $pK_a \sim 7.0$, conductivity decreases with uptake of protons. Dihydrogen phosphate–hydrogen phosphate buffer meets this need well with a $pK_a$ of 7.2 and a $\Delta \Lambda_0$ of $-78 \text{ S cm}^{-2} \text{ mol}^{-1}$:

$$\text{HPO}_4^{2-} + H^+ = \text{H}_2\text{PO}_4$$

The amine–sulphonic acid buffers of Good & Izawa (1972) also fit this case. They offer a choice of $pK_a$ values from 6.15 to 8.55 but no information is available on their $\Delta \Lambda_0$ values; e.g. BES [$N,N$-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid]:

$$(\text{HOCH}_2\text{H}_2)_2\text{NC}_2\text{H}_4\text{SO}_3 + H^+ = (\text{HOCH}_2\text{H})_2\text{NHC}_2\text{H}_4\text{SO}_3^+$$

(b) $pK_a \sim 7.0$, conductivity increases with uptake of protons. The only commonly used biological pH buffer in this category is Tris [tris(hydroxymethyl)aminomethane]:

$$(\text{HOCH}_2\text{H})_3\text{CNH}_2 + H^+ = (\text{HOCH}_2\text{H})_3\text{CNH}_3^+$$

However, its $pK_a$ is rather high at 8.3 and the uncharged conjugate base may be toxic. A compound that might be used at lower pH values is histidine, with a $pK_a$ of 6.0:

$$\text{N} - \text{NH} - \text{H} \overset{\text{C}}{\text{C}} \text{H} \overset{\text{C}}{\text{C}} \text{NH}_2 \overset{\text{C}}{\text{C}} \text{NH} - \text{COO}^- + H^+ \Rightarrow \text{N} - \text{NH} - \text{H} \overset{\text{C}}{\text{C}} \text{H} \overset{\text{C}}{\text{C}} \text{NH}_2 \overset{\text{C}}{\text{C}} \text{NH} - \text{COO}^-$$

(c) $pK_a \sim 4.5$, conductivity decreases with uptake of protons. A number of common carboxylic acids have $pK_a$ values in this region, e.g. oxalate, $pK_a = 4.28$, $\Delta \Lambda_0 = -108.2 \text{ S cm}^{-2} \text{ mol}^{-1}$:

$$-\text{OOCOO}^- + H^+ = \text{HOOCOO}^-$$

citrate, $pK_{a2} = 4.75$, $\Delta \Lambda_0 = ?$; $pK_{a3} = 5.45$, $\Delta \Lambda_0 = ?$:

$$\text{HOOCCH}_2\text{COHCOO}^- - \text{CH}_2\text{COO}^- + H^+ = \text{HOOCCH}_2\text{COHCOOHCH}_2\text{COO}^-$$

$$-\text{OOCCH}_2\text{COHCOO}^- - \text{CH}_2\text{COO}^- + H^+ = \text{HOOCCH}_2\text{COHCOO}^- - \text{CH}_2\text{COO}^-$$

However, in some cases their possible metabolism by microbes may be a disadvantage, and citrate tends to form metal complexes which have the effect of decreasing the net charge carried and hence the conducting power.

(d) $pK_a \sim 4.5$, conductivity increases with uptake of protons. There do not appear to be any obvious compounds to fulfill this role.

The concentration of pH buffer used in media should, of course, be adequate to absorb or release sufficient $H^+$ to prevent excessive pH drift. This might also assist in ‘locking’ the organisms into the desired metabolic pathway. It should also be appreciated that the $pK_a$ of acids in culture media may be different from the values given above, due to interionic interactions. If the ionic strength of the medium is known, appropriate corrections can be calculated (Butler, 1982).

$H^+$ flux

The net $H^+$ flux into or out of the cells determines the magnitude of the buffer-mediated conductivity change. Hence, as far as possible, nutrients should be selected, and metabolism directed, to promote the maximum flux of $H^+$ in one direction. For example, in a homolactic
Table 4. Effect of electron donor on $H^+$ flux and conductivity changes in microbial cultures

<table>
<thead>
<tr>
<th>Electron donor reaction</th>
<th>$H^+$ flux* [mol H$^+$ out (2e$^-$)$^{-1}$]</th>
<th>Net change in conductivity† [S cm$^2$ (2e$^-$)$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_2H_2O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$HCOO^- \rightarrow CO_2 + H^+ + 2e^-$</td>
<td>-1</td>
<td>-54.6</td>
</tr>
<tr>
<td>$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$</td>
<td>-0.25</td>
<td>-10.2</td>
</tr>
<tr>
<td>$C_2H_4COO^- + 6H_2O \rightarrow 4CO_2 + 19H^+ + 20e^-$</td>
<td>-0.1</td>
<td>-3.5</td>
</tr>
<tr>
<td>$(COO^-)_2 \rightarrow 2CO_2 + 2e^-$</td>
<td>-2</td>
<td>-148.4</td>
</tr>
<tr>
<td>$(CH_3COO^-)_2 + 4H_2O \rightarrow 4CO_2 + 12H^+ + 14e^-$</td>
<td>-0.29</td>
<td>-16.4</td>
</tr>
<tr>
<td>$C_2H_4NH_3^+ + 6H_2O \rightarrow 3CO_2 + NH_4^+ + 18H^+ + 18e^-$</td>
<td>0</td>
<td>+3.6</td>
</tr>
<tr>
<td>$H_2NCH_2COO^- + 2H_2O \rightarrow 2CO_2 + NH_4^+ + 5H^+ + 6e^-$</td>
<td>-0.33</td>
<td>+24.5</td>
</tr>
<tr>
<td>$C_6H_12O_6 \rightarrow 2CH_3CHOHCOO^- + 2H^+$</td>
<td>+1‡</td>
<td>+36§</td>
</tr>
<tr>
<td>$CH_3CHNH_3COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + NH_4^+ + 4H^+ + 4e^-$</td>
<td>0§</td>
<td>+57.2</td>
</tr>
<tr>
<td>$CH_3CHNH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCOO^- + NH_4^+ + 5H^+ + 4e^-$</td>
<td>+3§</td>
<td>+76.8</td>
</tr>
</tbody>
</table>

* All acids, other than CO$_2$, are assumed to be fully ionized.
† Net change in conductivity associated with oxidation of electron donor, expressed as change at infinite dilution per 2e$^-$ donated.
‡ Based on oxidation to pyruvate.
§ Estimated, taking $\lambda_0$ for lactate as 36 S cm$^{-2}$ mol$^{-1}$.
|| Retention of CO$_2$ as HCOO$^-$ has effects that are considered in detail later in the paper.

In fermentation the major $H^+$ flux is associated with excretion of lactic acid (i.e. outward flux of $H^+$, see Table 2) and additional outward $H^+$ flux might be obtained by the use of a positively charged fermentable substrate instead of glucose and by the use of NH$_4^+$ as nitrogen source rather than a neutral or negatively charged compound. A negatively charged fermentable substrate or nitrogen source should not be used since their uptake would be accompanied by uptake of $H^+$ in opposition to the excretion due to lactate production.

Electron donor

The electron donor must always be one of the major nutrients, and in fermentative cultures the electron donor and electron acceptor (which may, of course, be the same compound) are consumed in far greater quantities than other nutrients. Potentially, therefore, the electron donor has a major influence on conductivity changes in cultures.

Examples of oxidation reactions are shown in Table 4, with their associated $H^+$ fluxes and conductivity changes. These are expressed per 2-electron change in oxidation state because biological oxidations normally involve 2-electron transfers and because, for cultures generating ATP primarily by oxidative phosphorylation, the ATP generated and amount of growth supported are approximately proportional to the reducing power generated. Thus, presentation in this way gives a better guide to the relative conductivity changes to be expected per unit biomass synthesized than would presentation of changes per mole of substrate utilized. It is evident from Table 4 that: (a) oxidation of carboxylic acids to CO$_2$ leads to a decrease in conductivity whereas oxidation of amines or amino acids to CO$_2$ leads to an increase in conductivity due to release of NH$_4^+$; (b) the $H^+$ flux and conductivity change (per 2e$^-$) are higher for electron donors at a higher oxidation state than for those at a more reduced state (cf. the series HCOO$^-$ to C$_2$H$_4$COO$^-$); (c) Stickland-type oxidation of amino acids yields large increases in conductivity due to the production of organic acids and NH$_4^+$.

Electron acceptor

Various electron acceptor half-reactions are shown in Table 5, from which it is evident that there are considerable differences between the reactions both in $H^+$ fluxes and in conductivity.
Table 5. Effect of external electron acceptor on H⁺ flux and conductivity changes in microbial cultures

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>H⁺ flux [mol H⁺ out (2e⁻)⁻¹]</th>
<th>Net change in conductivity* [S cm² (2e⁻)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ + 4H⁺ + 4e⁻ → 2H₂O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO₂⁻ + 2H⁺ + 2e⁻ → NO + H₂O</td>
<td>0</td>
<td>(ΔκO₂⁻ - 71.4)</td>
</tr>
<tr>
<td>2NO₃⁻ + 12H⁺ + 10e⁻ → N₂ + 6H₂O</td>
<td>-0.4</td>
<td>-28.6</td>
</tr>
<tr>
<td>NO₂⁻ + 10H⁺ + 8e⁻ → NH₄⁺ + 3H₂O</td>
<td>-0.5</td>
<td>+0.5</td>
</tr>
<tr>
<td>S₂O₅²⁻ + 2e⁻ → 2SO₄²⁻</td>
<td>+2</td>
<td>+196.8</td>
</tr>
<tr>
<td>SO₄²⁻ + 8H⁺ + 8e⁻ → S²⁻ + 4H₂O</td>
<td>0</td>
<td>(ΔκSO₄²⁻ - 159.6)/4</td>
</tr>
<tr>
<td>CO₂ + 8H⁺ + 8e⁻ → CH₄ + 2H₂O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HCO₃⁻ + 9H⁺ + 8e⁻ → CH₄ + 3H₂O</td>
<td>-0.25</td>
<td>-11.1</td>
</tr>
<tr>
<td>(CH₃)₂NO + 3H⁺ + 2e⁻ → (CH₃)₂NH⁺ + H₂O</td>
<td>-1</td>
<td>+42</td>
</tr>
<tr>
<td>(CHCOO⁻)₂ + 2H⁺ + 2e⁻ → (CH₂COO⁻)₂</td>
<td>0</td>
<td>-9.0</td>
</tr>
<tr>
<td>H₂NCH₂COO⁻ + 2H⁺ + 2e⁻ → CH₂COO⁻ + NH₄⁺</td>
<td>0</td>
<td>+114.4</td>
</tr>
</tbody>
</table>

* Net change in conductivity at infinite dilution attributed to reduction of electron acceptor, expressed per 2 electrons accepted.

changes. Since many of the reactions shown are specific to particular microbial types, it is likely that these differences could be exploited in the design of culture media for the enumeration and/or identification of specific kinds of microbes or metabolic activities.

Carbon source

A difference between material assimilated and electron donor or acceptor compounds is that there are generally no products other than cell material. Hence, the only effect on medium conductivity is a decrease if the carbon source is charged or no change if it is not.

If a decrease in conductivity is desirable, it may be accentuated by the use of a multi-charged compound with a high charge-to-mass ratio, since such compounds tend to exhibit higher molar conductivities on a per charge basis than do compounds bearing only one charge (Table 1). However, this advantage may be lessened by a countervailing greater tendency of the multi-charged compounds to ion-pair formation or metal chelation.

In cultures where a net increase in conductivity is expected the situation is more complex. Any charged compound taken up by cells influences the conductivity of the medium by two routes. Firstly, there is an inevitable reduction in conductivity due to the removal of charged ions from solution. Secondly, there is a change in the conductivity of the ions of the pH buffer due to H⁺ flux associated with uptake of the charged nutrient. Hence, preference for a neutral or a charged carbon source depends upon whether the net result of these two effects is an increase or a decrease in conductivity.

Nitrogen source

The main types of compounds used as nitrogen sources are listed in Table 6 along with the associated H⁺ fluxes and conductivity changes. Values for the molar conductivities of the organic nitrogen sources do not appear to be available, but it may be assumed that, due to the larger sizes of the molecules, they will be considerably less than the values for NH₄⁺ and NO₃⁻. Hence, conductivity decrease may be maximized by the use of NH₄⁺ or NO₃⁻, whereas if no conductivity decrease is desired, an amino acid zwitterion with no net charge may be appropriate.

Metabolic products

The general effects of different metabolic products on H⁺ flux and conductivity changes have already been indicated in the discussions on electron donors, electron acceptors, carbon sources and nitrogen sources. However, one aspect not so far considered is the fate of CO₂, if produced,
Table 6. Effect of nitrogen source on H\(^+\) flux and conductivity changes in microbial cultures

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>H(^+) flux (mol H(^+) out (mol N-source)(^{-1}))</th>
<th>Change in conductivity* (S cm(^{-2}) mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH(_3)</td>
<td>1</td>
<td>-73.5</td>
</tr>
<tr>
<td>N(_2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO(_3)</td>
<td>-1</td>
<td>-71.4</td>
</tr>
<tr>
<td>H(_2)NCH(_2)COO(^-)</td>
<td>0†</td>
<td>0†</td>
</tr>
<tr>
<td>-OOCCH(_2)CHNH(_2)COO(^-)</td>
<td>-1†</td>
<td>-(\lambda)(_{\text{kap}})†</td>
</tr>
<tr>
<td>H(_3)N(CH(_2))(_2)CHNH(_3)COO(^-)</td>
<td>1†</td>
<td>-(\lambda)(_{\text{kap}})†</td>
</tr>
</tbody>
</table>

* Net change in conductivity at infinite dilution attributed to assimilation of nitrogen source.
† Assumes that the carbon moiety is assimilated.

Table 7. Calculated concentrations of CO\(_2\), HCO\(_3\) and CO\(_3\)\(^-\) present in solutions of different pH values in equilibrium with air

The concentrations were calculated taking \(P_{\text{CO}_2}\) in air = 10\(^{-3.5}\) atm, \(K_H = 10^{-1.47}\), \(K_1 = 10^{-6.35}\), and \(K_2 = 10^{-10.33}\) at 25°C.

<table>
<thead>
<tr>
<th>pH</th>
<th>[CO(_2)] (mol l(^{-1}))</th>
<th>[HCO(_3)] (mol l(^{-1}))</th>
<th>[CO(_3)(^-)] (mol l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.07 \times 10(^{-5})</td>
<td>4.8 \times 10(^{-9})</td>
<td>2.3 \times 10(^{-16})</td>
</tr>
<tr>
<td>5</td>
<td>1.07 \times 10(^{-5})</td>
<td>4.8 \times 10(^{-7})</td>
<td>2.2 \times 10(^{-12})</td>
</tr>
<tr>
<td>7</td>
<td>1.07 \times 10(^{-5})</td>
<td>4.8 \times 10(^{-5})</td>
<td>2.2 \times 10(^{-8})</td>
</tr>
<tr>
<td>8</td>
<td>1.07 \times 10(^{-5})</td>
<td>4.8 \times 10(^{-4})</td>
<td>2.2 \times 10(^{-6})</td>
</tr>
<tr>
<td>9</td>
<td>1.07 \times 10(^{-5})</td>
<td>4.8 \times 10(^{-3})</td>
<td>2.2 \times 10(^{-4})</td>
</tr>
<tr>
<td>10</td>
<td>1.07 \times 10(^{-5})</td>
<td>4.8 \times 10(^{-2})</td>
<td>2.2 \times 10(^{-2})</td>
</tr>
</tbody>
</table>

and its possible effects on H\(^+\) flux and conductivity change. There are three possible fates for CO\(_2\) produced within cultures: (a) it is released into the atmosphere, (b) it remains in solution as dissolved CO\(_2\), (c) it is hydrolysed partially or completely to bicarbonate: CO\(_2\) + H\(_2\)O \rightleftharpoons HCO\(_3\) + H\(^+\). Fates (a) and (b) have no effect on H\(^+\) flux or conductivity but formation of HCO\(_3\) has effects on both. Which of these three fates befalls the CO\(_2\) in cultures depends upon whether the system is sealed or open to the atmosphere and upon the pH value of the medium.

**CO\(_2\) equilibria in media open to the atmosphere.** The basic relationships at equilibrium are described by Butler (1982):

\[
[\text{CO}_2] = K_H P_{\text{CO}_2} = 10^{-4.97}
\]

\[
[\text{HCO}_3^-] = \frac{K_C C_H P_{\text{CO}_2}}{[H^+]} = 10^{-11.32}
\]

\[
[\text{CO}_3^{2-}] = \frac{K_S C_C C_H P_{\text{CO}_2}}{[H^+]^2} = 10^{-21.65}
\]

where [CO\(_2\)] is the concentration of CO\(_2\) in solution (mol l\(^{-1}\)) (some CO\(_2\) is present as H\(_2\)CO\(_3\), but since [H\(_2\)CO\(_3\)] is only 10\(^{-3}\) of [CO\(_2\)], [CO\(_2\)]+ [H\(_2\)CO\(_3\)] is referred to as [CO\(_2\)], \(P_{\text{CO}_2}\) is the partial pressure of CO\(_2\) in the atmosphere (10\(^{-3.5}\) atm in air), \(K_H\) is Henry’s Law constant (10\(^{-1.47}\) mol l\(^{-1}\) atm\(^{-1}\) at 25°C), [HCO\(_3\)] is the concentration of HCO\(_3\) in solution, [CO\(_3\)\(^-\)] is the concentration of CO\(_3\)\(^-\) in solution, \(K_C\) is the first dissociation constant for carbonic acid (10\(^{-6.35}\) at 25°C), and \(K_S\) is the second dissociation constant for carbonic acid (10\(^{-10.33}\) at 25°C).

Thus the concentration of CO\(_2\) in solution is independent of pH while the concentrations of HCO\(_3\) and CO\(_3\)\(^-\) are pH dependent. Nevertheless, it is evident that the concentrations of all
Table 8. Effect of pH on the proportions of total carbonate present as CO\(_2\), HCO\(_3^-\) and CO\(_3^{2-}\) in solution in a closed container without a gas phase

<table>
<thead>
<tr>
<th>pH</th>
<th>CO(_2)</th>
<th>HCO(_3^-)</th>
<th>CO(_3^{2-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>~1.0</td>
<td>~0</td>
<td>~0</td>
</tr>
<tr>
<td>4</td>
<td>~1.0</td>
<td>~0</td>
<td>~0</td>
</tr>
<tr>
<td>5</td>
<td>0.96</td>
<td>0.04</td>
<td>~0</td>
</tr>
<tr>
<td>6</td>
<td>0.69</td>
<td>0.31</td>
<td>~0</td>
</tr>
<tr>
<td>7</td>
<td>0.18</td>
<td>0.82</td>
<td>~0</td>
</tr>
<tr>
<td>8</td>
<td>0.02</td>
<td>0.97</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>~0</td>
<td>0.95</td>
<td>0.05</td>
</tr>
<tr>
<td>10</td>
<td>~0</td>
<td>0.68</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Calculated taking \(K'_1 = 10^{-6.15}\), \(K'_2 = 10^{-10.33}\) at 25 °C.

three in equilibrium with the concentration of CO\(_2\) in air are so low at the usual pH values of microbial cultures that the contributions of HCO\(_3^-\) and CO\(_3^{2-}\) to H\(^+\) flux and conductivity changes will generally be negligible (Table 7). However, it should be borne in mind that culture media may not be in equilibrium with atmospheric CO\(_2\), or may not attain equilibrium rapidly.

**CO\(_2\) equilibria in media in closed vessels without a gas phase.** Under these conditions, the concentrations of the different species are given by the following relationships (Butler, 1982):

\[
[CO_2] = C_T^C \left( \frac{[H^+]^2}{K'_1 K'_2 + K'^C[H^+] + [H^+]^2} \right)
\]

\[
[HCO_3^-] = C_T^C \left( \frac{K'^C[H^+]}{K'_1 K'_2 + K'^C[H^+] + [H^+]^2} \right)
\]

\[
[CO_3^{2-}] = C_T^C \left( \frac{K'_1 K'_2}{K'_1 K'_2 + K'^C[H^+] + [H^+]^2} \right)
\]

where \(C_T^C\) is the total concentration of carbonate present (i.e. \([CO_2] + [HCO_3^-] + [CO_3^{2-}]\)). Thus, for a given pH value, the amount of each species is a constant proportion of the total carbonate carbon present. These proportions, and how they change with pH, are shown in Table 8. It is clear that CO\(_3^{2-}\) can generally be ignored in cultures and that, so far as effects on conductivity and H\(^+\) flux are concerned, only the concentration of HCO\(_3^-\) is of consequence. The pH of cultures following the production of a given amount of CO\(_2\) is calculated as indicated earlier, and appropriate allowance is then easily made for the conductivity due to HCO\(_3^-\).

**CO\(_2\) equilibria in media in closed vessels with a gas phase.** For a closed vessel containing \(V_L\) litres of culture medium with a concentration of CO\(_2\) in solution of \([CO_2]_L\) mol l\(^{-1}\), and a gas phase of \(V_G\) litres with a partial pressure of CO\(_2\) of \(P_{CO_2}\) atm, the basic relationships are:

\[
[CO_2]_L = K_H P_{CO_2}
\]

\[
[CO_2]_L = \frac{[HCO_3^-][H^+]}{K'^C}
\]

\[
C_T = C'_G^{CO_2} + C'_L^{CO_2} + C'_L^{HCO_3^-} + C'_L^{CO_3^-}
\]
where \( C_T \) is the total amount of \( \text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-} \) in the vessel, \( C^\text{CO}_2 \) is the amount of \( \text{CO}_2 \) in the gas phase (mol), \( C^\text{HCO}_3^- \) is the amount of \( \text{CO}_3^- \) in solution (mol), and \( C^\text{CO}_3^{2-} \) is the amount of \( \text{CO}_3^{2-} \) in solution (mol). Thus:

\[
C_T = P_{\text{CO}_2} \frac{V_G}{V_m} + [\text{CO}_2]_L V_L + [\text{HCO}_3^-] V_L + [\text{CO}_3^{2-}] V_L
\]

\[
P_{\text{CO}_2} = \frac{[\text{HCO}_3^-][\text{H}^+]}{K_i C_H}
\]

For pH below 9, \( \text{CO}_3^{2-} \) can be neglected, and:

\[
C_T = \frac{[\text{HCO}_3^-][\text{H}^+]}{V_m K_i C_H} + \frac{[\text{HCO}_3^-][\text{H}^+] V_L}{K_i} + [\text{CO}_3^-] V_L
\]

where \( V_m \) is the molar volume of an ideal gas at 1 atm and 25 ℃ (24.47 l mol\(^{-1}\)).

Dividing by \( V_L \):

\[
\frac{C_T}{V_L} = \frac{[\text{HCO}_3^-][\text{H}^+]}{V_m K_i C_H} + \frac{[\text{HCO}_3^-][\text{H}^+] V_L}{K_i} + [\text{CO}_3^-]
\]

If we put \( V_L = 1 \) and \( V_G/V_L = V_R \), where \( V_R \) is the ratio of volume of gas phase to volume of liquid phase in the vessel, then:

\[
C_T = [\text{HCO}_3^-] \left( \frac{[\text{H}^+] V_R}{V_m K_i C_H} + \frac{[\text{H}^+]}{K_i} + 1 \right)
\]

and

\[
[HCO_3^-] = C_T \left( \frac{V_m K_i C_H}{V_m K_i C_H + V_m K_H [H^+] + [H^+] V_P} \right)
\]

where \( \alpha \) equals the term in round brackets.

And, since \( V_L = 1 \) and \( V_G = V_R \):

\[
C^\text{HCO}_3^- = [\text{HCO}_3^-]
\]

\[
C^\text{CO}_2 = [\text{CO}_2]_L = \frac{[\text{HCO}_3^-][\text{H}^+]}{K_i}
\]

\[
C^\text{CO}_3^- = C_T - C^\text{CO}_2 - C^\text{HCO}_3^-
\]

\[
= C_T - [\text{HCO}_3^-] \left( \frac{[\text{H}^+]}{K_i} + 1 \right)
\]

And the proportions of the total represented by each species are given by:

\[
C^\text{HCO}_3^-/C_T = \alpha
\]

\[
C^\text{CO}_2/C_T = \alpha ([\text{H}^+]/K_i
\]

\[
C^\text{CO}_3^-/C_T = 1 - \alpha ([\text{H}^+]/K_i) + 1
\]

The calculated relative proportions of each species for different gas volume to liquid volume ratios are shown in Table 9. It is evident that, even with a large gaseous phase, a substantial proportion (25% at pH 7 with a gas:liquid ratio of 10:1) of the total carbonate in a closed container can be in the form of \( \text{HCO}_3^- \) if the pH is near or above neutrality.

**Strategies for \( \text{CO}_2/\text{HCO}_3^- \) production in culture media.** Apart from the direct contribution of \( \text{HCO}_3^- \) to conductivity, the formation of \( \text{HCO}_3^- \) is accompanied by the production of \( \text{H}^+ \) (i.e.
### Table 9. Effect of ratio of volume of gas phase to volume of liquid phase and pH value on the relative proportions of gaseous CO₂, dissolved CO₂ and HCO₃⁻ in a closed vessel

<table>
<thead>
<tr>
<th>pH</th>
<th>Volume of gas phase/volume of liquid</th>
<th>Proportion of total CO₂ as*:</th>
<th>Dissolved CO₂</th>
<th>Gaseous CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HCO₃⁻</td>
<td>CO₂</td>
<td>CO₂</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>~0</td>
<td>0.03</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>0.08</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.01</td>
<td>0.21</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.02</td>
<td>0.44</td>
<td>0.54</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0.01</td>
<td>0.03</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.03</td>
<td>0.08</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.09</td>
<td>0.20</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.17</td>
<td>0.38</td>
<td>0.54</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>0.11</td>
<td>0.02</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.25</td>
<td>0.06</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.49</td>
<td>0.11</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.67</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>0.55</td>
<td>0.01</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.77</td>
<td>0.02</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.91</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.95</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Calculated as described in the text, taking $K_H = 10^{-14.7}$, $K'_H = 10^{-6.35}$ and $V_m = 24.47 \text{ mol}^{-1} \text{ at 1 atm and 25 } \degree \text{C}$. 

outward H⁺ flux). Hence, if the major H⁺ flux is inward, then retention of CO₂ as HCO₃⁻ is not desirable and the culture should be open to the atmosphere and/or maintained at a pH near or below 5.

If the major anticipated H⁺ flux is outward, retention of CO₂ as HCO₃⁻ is desirable to accentuate the flux. This may be achieved by the following means.

(a) Keeping cultures tightly sealed to prevent escape of CO₂ to the atmosphere.

(b) Maintaining the pH of cultures near neutrality or higher, when virtually all the dissolved carbonate will be present at HCO₃⁻ (Table 8).

(c) Providing the smallest possible gas space in anaerobic cultures. Since production of CO₂ leads to an increase in pressure, it may be advisable to restrict the initial nutrient concentrations to control this. For example, the generation of 0.1 M-CO₂ in a sealed vessel without a gas phase would exert a partial pressure of nearly 3 atm at 25 °C at pH values below about 5.

(d) Providing the minimum gas phase in aerobic cultures that is commensurate with the provision of sufficient O₂ for growth. For example, the complete oxidation of 0.75 g glucose l⁻¹ requires approximately 3 volumes of air per volume of medium at 25 °C. At pH 7 half of the CO₂ formed would be in solution in the medium as HCO₃⁻ (Table 9). Hence, it would be useful to use a closed vessel containing this proportion of air providing that the culture pH could be kept near neutrality or higher. With 7.5 g glucose l⁻¹, 30 volumes of air are required per volume of medium and the proportion of the CO₂ that remains in solution as HCO₃⁻ is then much less unless the pH value is near 8 or above. Nevertheless, in the absence of other sources of conductivity changes, the retention of even a small proportion of the CO₂ as HCO₃⁻ might be sufficient to generate a detectable conductivity change. In aerobic cultures, pressure changes are not liable to present any hazard since O₂ is consumed and partially replaced by CO₂.

These strategies for maximizing the conductivity changes by retention of CO₂/HCO₃⁻ in cultures assume that the microbial metabolism is unaffected by high concentrations of these compounds. It should be appreciated that this may not always be the case and that changes in the metabolic products formed could nullify or enhance the effects sought.

**Other inorganic nutrients**

The other inorganic nutrients consumed by cells during growth are required in relatively small amounts and their consumption has a correspondingly small effect on the overall conductivity...
change (Table 2). Hence, little consideration needs to be given to the selection of the particular salts. However, to reduce ion-pair formation effects it may be worthwhile to use uni-univalent salts rather than uni-bivalent salts where possible.

Other metabolic activities

The major activity to be considered under this heading is the breakdown of polymers. Increases in the conductivity of cultures are commonly attributed to the breakdown of polymers to smaller molecules (e.g. Eden & Eden, 1984) but the precise mechanisms involved are not obvious.

In the case of sugar polymers hydrolysis will yield only uncharged sugar molecules and no change in conductivity would be anticipated. With a polymer such as pectin (polygalacturonic acid) an increase in conductivity would occur on hydrolysis due to the greater mobility of the monomer molecules. However, its extent would depend upon the degree to which monomers accumulate or are metabolized by the microbes.

Similarly, the hydrolysis of triglyceride fats to glycerol and free fatty acids would be expected to lead to an increase in conductivity. However, there do not appear to be reports of studies on conductivity changes associated with fat metabolism.

The polymers whose breakdown has received most attention are the proteins and peptides that occur in most complex microbiological culture media. These compounds bear some net charge themselves and thus make a contribution to carrying current, but since the molecules are large, and have relatively small net charges at the pH values of cultures, this contribution is small. Hydrolysis to individual amino acids will probably lead to some increase in conductivity, but the increase may not be large since most amino acids are zwitterions of the form H, NRCOO– and bear no net charge at pH values about 4 to 8 (Mahler & Cordes, 1971). The only amino acids carrying net charges in this pH range are cysteine, cystine and the dicarboxylic and basic ones, namely glutamate, aspartate, lysine, hydroxylysine, arginine and histidine. It follows that conductivity changes due to polypeptide hydrolysis might be maximized by the use of proteins rich in these amino acids, or of appropriate synthetic polypeptides. However, in vigorously growing cultures the amino acids may not accumulate, due to their rapid utilization by the microbes. In such a case, nitrogen that is surplus to biosynthetic needs will be excreted into the medium as NH₄⁺, with a concomitant increase in medium conductivity. When amino acids serve as carbon sources and electron donors in aerobic metabolism the excretion of NH₄⁺ must be substantial (Table 4) and it is even greater with microbes, such as the clostridia, that are able to ferment amino acids. In the latter case the increase in conductivity is further enhanced by the formation of organic acids (Tables 4 and 5). Parsons & Sturges (1926) suggested that the increase in conductivity observed in clostridial cultures could be entirely accounted for by the NH₄⁺ formed. Thus, it seems very probable that the major mechanism by which increases in conductivity occur in carbohydrate-free peptone-containing culture media involves the formation of highly mobile NH₄⁺ ions, rather than the production of largely non-conducting amino acid molecules. It follows that the amount of NH₄⁺ excreted during aerobic metabolism, and the conductivity increase, may be maximized by the use of polypeptides or amino acids with high nitrogen-to-carbon ratios.

General considerations

Apart from the nature of the specific metabolic activities of the microbes, the conductivity of ions in solution is influenced by a number of general factors. The effects of these factors may be small or entirely negligible or may not be amenable to manipulation by culture medium formulation. Nevertheless, they should be borne in mind when culture media for conductimetric assays are formulated.

(a) The molar conductivities of small ions, with some exceptions among very small ones, are normally greater on a per charge basis than those of larger ions (Table 1) and in the absence of specific information on the conductivities of compounds this relationship can be assumed.

(b) The molar conductivities of multi-charged ions are normally proportionately greater on a per charge basis than those of uni-charged compounds (Table 1). This suggests that, where a
large conductance change is desired, it would be advantageous, for example, to use a $B^3-/BH^2-$ or $B^2-/BH-$ rather than a $B^-/BH^0$ buffer compound.

(c) Ion-pair formation, and the consequent removal of ions from current carrying, is greater with multi-charged ions than with singly-charged ones. Thus, once the nutritional requirements for sulphur and phosphorus are satisfied, metal salts should preferably be supplied as chlorides rather than as sulphates or phosphates. The possible occurrence of ion-pair formation also suggests that, in some cases, a $B^-/BH^0$ buffer might yield a larger conductivity change than $B^2-/BH-$ or $B^3-/BH^2-$ buffers. This contradicts the suggestion above that larger conductivity changes would be expected with multi-charged buffers than with singly-charged ones. In practice, it is not easy to predict whether the benefits of multiple charges will be outweighed by ion-pair formation. However, since data on the molar conductivities at infinite dilution of buffers are available, or may be determined, whereas the effects of ion-pair formation are not readily evaluated, it would seem preferable to base the selection of buffers initially on their molar conductivities at infinite dilution. In addition, large multi-charged molecules may chelate metal ions, and similar kinds of considerations apply as just discussed for ion-pair formation.

(d) The higher the total ionic strength of the medium, the greater is the depression of the molar conductivities of the constituent ions from their values at infinite dilution. Hence, absolute and percentage changes in conductivity will be greater in media of low ionic strength than in those of high ionic strength. The ionic strength of media could be minimized by using minimal concentrations of nutrients and by using singly-charged ions in preference to multi-charged ones.

(e) Viscosity has a major effect on the mobilities of ions in solution and hence on the conductivity of solutions. However, the effect is small at the molecular concentrations existing in most culture media, and the effects on conductivity of changes in viscosity during microbial growth are likely to be negligible. The addition of low concentrations (about 0.1–0.2%) of agar to liquid culture media is helpful to stabilize media–test sample mixtures in cases where the slow sedimentation of particles or separation of emulsions would cause base-line drift or product interference with the conductance signal independent of microbial growth (Malthus Instruments, personal communication). Such concentrations of agar have a negligible effect on the conductivity of culture media (author's unpublished observations). Curtis et al. (1985) recommended the use of dextran, $4\text{ g}\text{l}^{-1}$, in blood cultures to eliminate the signal due to erythrocyte sedimentation. They reported that neither 4 nor 40 g dextran l$^{-1}$ had any significant effect on the rates of conductivity changes in broth cultures.

RATE OF CHANGE OF CONDUCTIVITY WITH TIME

The preceding discussions have concentrated upon ways of maximizing the total change in conductivity associated with microbial growth without giving attention to whether the change occurs rapidly or slowly. However, in routine conductimetric microbiological assays speed is important.

The rate of conductivity change in a growing culture will, other things being equal, depend upon both the net conductivity change per unit biomass produced and the rate of growth of that biomass. Microbial growth is generally fastest in complex media which contain many nutrients in ready-made form. Such media are inherently relatively non-specific with regard to the metabolic activities that can occur, whereas simpler media may 'lock' an organism into a particular type of metabolism. Hence, there is a potential conflict in the formulation of media for conductimetric assays between a desire to use complex media to promote rapid growth and a desire to use simple media that 'lock' the microbes into pathways that maximize conductivity changes. The exact balance between these two ideals would depend upon the particular application and priorities.

EXPERIMENTAL STUDIES

The experimental investigation of the mechanisms of conductivity changes in microbial cultures depends upon having sufficiently simple and well-defined systems in which the
metabolic activities of the microbes are controlled and known. Such an approach was used by Owens et al. (1985) in a study on the conductivity changes during the oxidation of formate by non-growing cell suspensions of *Escherichia coli* with trimethylamine oxide as final electron acceptor. That the metabolism was 'locked' into the intended reactions was shown by the close agreement between observed and predicted changes in pH. Clear evidence was obtained that a major proportion of the conductivity change associated with the oxidation-reduction was due to the conversion of dihydrogen phosphate buffer to hydrogen phosphate, as predicted from theoretical considerations. Hence, the work of Owens et al. (1985) supports the validity of the general approach and model presented in this paper. In particular, it confirms the thesis that the pH buffer is potentially one of, if not the most, important sources of conductivity changes in microbial cultures and emphasizes that selection of an appropriate pH buffer system is crucial in attempts to maximize conductivity changes in cultures.

An effect of the pH buffer has also been reported by Malthus Instruments (personal communication). During the development of the Malthus Coliform Medium it was noted that the conductivity change produced by microbial growth was greater in the presence of Tris buffer than with phosphate buffer. This is readily understood in lactose-fermenting and acid-producing cultures. As pointed out above, the conductivity of Tris buffer increases with decline in pH and thus aids the increase in conductivity due to the formation of acids, whereas the conductivity of phosphate decreases with decline in pH and counteracts the increase associated with acid production. The data presented in Table 10 are also in accordance with this explanation. The fermentation of glucose by *E. coli* in a mineral salts medium containing phosphate buffer was accompanied by a decrease in conductivity of the medium whereas an increase in conductivity occurred with Tris or histidine buffers.

Comparison of experimentally observed changes in the conductivity of cultures in different media with the changes predicted by calculation (Table 10) shows that all the observed changes are in the predicted directions and that their magnitudes are substantially less than the calculated values. The observed changes are expected to be less than theoretical changes calculated using molar conductivities at infinite dilution, for the reasons indicated previously, but the extent of this difference cannot be estimated precisely. The conductivity changes observed range from 51 to 78% of the calculated values and would appear to be reasonable for media having a total ion concentration of 0.05–0.07 mol l⁻¹. In those cases where the discrepancy is especially large, it is probable that this is due to the formation of metabolic products different from those assumed in the calculations. For future studies, accuracy might be improved by using media containing a minimum of multi-charged ions, to minimize interionic interactions, and assaying the metabolic products formed in the cultures.

### Table 10. Experimentally determined and theoretical changes in conductivity of cultures of *E. coli* K12 grown in different culture media

Cultures were grown in a defined mineral salts medium containing 0.02 M-buffer and sufficient carbon source to support approximately 100 mg dry biomass l⁻¹.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Growth conditions</th>
<th>pH buffer</th>
<th>Change in conductivity (µS·cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Aerobic</td>
<td>Phosphate</td>
<td>Predicted*</td>
</tr>
<tr>
<td>Glucose</td>
<td>Anaerobic</td>
<td>Phosphate</td>
<td>-180</td>
</tr>
<tr>
<td>Glucose</td>
<td>Anaerobic</td>
<td>Tris</td>
<td>+ ?†</td>
</tr>
<tr>
<td>Glucose</td>
<td>Anaerobic</td>
<td>Histidine</td>
<td>+ ?†</td>
</tr>
<tr>
<td>Alanine</td>
<td>Aerobic</td>
<td>Phosphate</td>
<td>+360</td>
</tr>
</tbody>
</table>

* Predicted values were calculated as described in the text and are corrected for the approximate proportions of CO₂ and HCO₃⁻ existing in the cultures at their final pH values.
† An increase in conductivity is predicted but cannot be quantified due to lack of data on the molar conductivity of the buffer.
CONCLUSIONS

To date, the selection of culture media for conductimetric microbiological assays has been an empirical, trial-and-error procedure, expensive in both time and effort. Even when an apparently satisfactory medium is found, its reliability cannot be assumed in all circumstances, due to lack of knowledge of the mechanisms involved. Since conductivity changes can differ between even closely related reactions (Owens et al., 1985), highly specific conductimetric assays cannot be designed without a full understanding of the sources of conductivity changes in cultures.

The model of conductivity changes in microbial cultures presented in this paper allows the direction and relative rates of the changes to be predicted from theoretical calculations. This should permit the rational design of media for the enumeration of specific categories of microbes, for the identification of species, or for assessment of particular enzyme activities. The development of such media, combined with the simplicity of the basic methodology, will ensure the increasing application of conductimetric techniques to routine microbiological and biochemical assays.

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REFERENCES


