The inhibition by \( \text{CO}_2 \) of the growth and metabolism of micro-organisms

Neil M. Dixon & Douglas B. Kell* Department of Biological Sciences, University College of Wales, Aberystwyth, Dyfed, SY23 3DA, UK

Received 17 August 1988

1. Introduction, 109
2. \text{CO}_2; concentrations, 110
   2.1. Dissolved \text{CO}_2; concentration, 110
   2.2. Bicarbonate concentration in a pH-controlled culture with a constant temperature and gas phase, 110
3. Historical, 111
4. Treatment of water with \text{CO}_2, 111
5. Treatment of dairy products with \text{CO}_2, 113
6. Preservation of meat and fish by \text{CO}_2, 114
   6.1. Inhibition of the growth and metabolism of food-spoilage micro-organisms, 114
   6.2. \text{CO}_2; resistance of food-related bacteria, 116
   6.3. \text{CO}_2; in the prevention of food spoilage by clostridia, 116
   6.4. Problems associated with the preservation of meat by \text{CO}_2; containing atmospheres, 116
   6.5. Preservation of seafood by \text{CO}_2; containing atmospheres, 117
7. Preservation of fruit and vegetables by \text{CO}_2, 118
8. Control of the production of fermented foods and beverages using \text{CO}_2, 119
9. Inhibition of other industrial fermentation processes by \text{CO}_2, 119
   9.1. Effects of \text{CO}_2; on the production of biomass, 119
   9.2. Effects of \text{CO}_2; on the production of biochemicals, 121
   9.3. Effects of \text{CO}_2; on the treatment of wastes, 121
10. Sites of action, 122
    10.1. Biological membranes, 122
        10.1.1. The metabolic control analysis, 123
    10.2. Cytoplasmic enzymes as a site of action of \text{CO}_2, 124
        10.2.1. \text{CO}_2; controlled induction/repression of enzyme synthesis, 124
        10.2.2. \text{CO}_2; inhibition of enzyme reactions, 124
11. Mechanisms of \text{CO}_2; inhibition of microbial growth and metabolism, 125
12. Concluding remarks, 127
13. Acknowledgements, 127
14. References, 128

1. Introduction

It has been known for many years that the growth and metabolism of micro-organisms is accompanied by the uptake and/or evolution of carbon dioxide. Yet, except for the obvious case of autotrophic micro-organisms, the partial pressure of \( \text{CO}_2 \) (\( p\text{CO}_2 \)) has rarely been considered to be of much quantitative significance to the physiology of the cell. Although there have been a number of reviews on the effects of \( \text{CO}_2 \) on the requirement for and fixation of \( \text{CO}_2 \) by micro-organisms, especially autotrophic ones (e.g. Fuchs & Stupperich 1983; Codd 1984; Fuchs 1986), in general little attention appears to have been concentrated on what are in fact a rather widespread set of studies concerning

* Corresponding author.
CO₂ inhibition. Nonetheless, CO₂ is inhibitory to the growth of a number of micro-organisms, a fact which has enjoyed increasing exploitation in the preservation of foodstuffs from bacterial spoilage. It therefore seemed appropriate to collate and summarize this material, a survey which forms the subject matter of the present review. After an historical introduction, the use of CO₂ as an antimicrobial agent for use in the preparation of potables and consumables is considered. This leads naturally to a review of some of the antimicrobial properties of CO₂ during industrial fermentations. Many of these observations have been of a somewhat empirical nature; thus, finally we consider some of the more molecular or mechanistic studies which have sought to ascertain the means by which CO₂ might be acting to cause microbial growth inhibition. We begin with a brief discussion of the thermodynamics of the interactions of CO₂ and aqueous media.

2. 'CO₂' concentrations

Although the partial pressure of CO₂ in the gas phase may be held constant, the ratios of the different possible species of 'CO₂' in the aqueous phase will vary as a function of the pH and other factors. Since CO₂ can hydrate and dissociate in water, the reaction scheme may be written as (Knoche 1980):

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CO}_3^{2-} + 2\text{H}^+
\]  

(1)

In addition it has recently been proposed that small concentrations of dimeric hydrogen carbonate ions (\(\text{H}_3\text{C}_2\text{O}_4^-\)) exist near neutral pH (Covington 1985). Since the concentration of this species is negligible, however, such ions will not be considered in the following. At pH values less than 8, the concentration of carbonate ions may be neglected (Yagi & Yoshida 1977) and only the following hydration reactions need to be considered:

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

(2)

2.1. DISSOLVED CO₂ CONCENTRATION

The concentration of CO₂ in solution ([CO₂]aq) is normally expressed by Henry's law (Butler 1982; Ho et al. 1987):

\[
[\text{CO}_2]_{\text{aq}} = K_H p\text{CO}_2
\]

(3)

where \(K_H\) = the Henry's law constant (in units of mol/l atm) and \(p\text{CO}_2\) = the partial pressure of CO₂ in the gas phase (in atm). For cultures grown under atmospheric pressure, the proportionality of solubility and partial pressures (Henry's law) may be assumed without introducing appreciable errors (Schumpe et al. 1982).

At a temperature of 37°C, \(K_H = 10^{-1.61}\) (Butler 1982) where the \([\text{CO}_2]\) is expressed in molar terms. Thus to obtain \([\text{CO}_2]\) in millimolar terms, \(K_H = 10^{1.39}\). Hence:

\[
[\text{CO}_2]_{\text{aq}} = 10^{1.39} \times p\text{CO}_2 = 24.6 \times p\text{CO}_2
\]

In other words, when \(p\text{CO}_2 = 1 \text{ atm}\), the concentration of dissolved CO₂ = 24.6 mmol/l.

2.2. DICARBONATE CONCENTRATION IN A pH-CONTROLLED CULTURE WITH A CONSTANT TEMPERATURE AND GAS PHASE

The equilibrium between CO₂ and HCO₃⁻ is defined by a 'hybrid' equilibrium constant \(K'_1\) (Butler 1982) where

\[
K'_1 = \frac{10^{-1\text{H}} \times [\text{HCO}_3^-]}{[\text{CO}_2]}
\]

(4)

From eqn (4) it follows that:

\[
\log_{10}[\text{HCO}_3^-] = \text{pH} - pK'_1 + \log_{10}[\text{CO}_2]
\]

(5)
\textit{CO}_2 \textit{inhibition}  \\
pK'_1 \text{ is related to the thermodynamic pK of the reaction } pK^0_1 \text{ and the ionic strength } l \text{ by:  \\
pK'_1 = pK^0_1 - 0.5f(l) - bl}  \\
\text{From Davies's equation (Butler 1982),  \\
f(l) = [1^{1/2}/(1 + 1^{1/2}) - 0.21][(298)/(T + 273)]^{2/3}  \\
\text{where } T \text{ is the temperature in } ^\circ\text{C}, \text{ } l \text{ is the ionic strength of the medium and is given by  \\
l = 1/2 \sum c_i z_i^2}  \\
\text{where } c_i \text{ = the concentration of ion } i \text{ and } z_i \text{ = the charge on ion } i.  \\
\text{To obtain the apparent } pK_{a,1} \text{ for the } CO_2/HCO^-_3 \text{ equilibrium, we use (Butler 1982)  \\
pK_{a,1} = pK^0_1 - f(l) - bl}  \\
\text{Therefore from eqn (9) we obtain } pK_{a,1}, \text{ and so from eqn (5)  \\
\log_{10}[HCO^-_3] = pH - pK_{a,1} + \log_{10}[CO_2].}  \\
\text{A typical value of } pK_{a,1} \text{ is 6.3 (at zero ionic strength and 35}^\circ\text{C, Butler 1982). Thus the addition of } CO_2 \text{ to an aqueous medium will tend to cause the pH to drop to a value approximately equal to the } pK, \text{ depending upon the other buffering constituents in the medium.}
Table 1. The effect of pH and CO₂ on the growth of micro-organisms (Hays et al. 1959)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Controls pH</th>
<th>Growth</th>
<th>Samples pH</th>
<th>Growth</th>
<th>Nitrogen Controls pH</th>
<th>Growth</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella aerogenes</td>
<td>6-7</td>
<td>+ +</td>
<td>5-6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>6-9</td>
<td>+ +</td>
<td>5-4</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium butyricum</td>
<td>5-5</td>
<td>+ +</td>
<td>5-0</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>6-9</td>
<td>+ +</td>
<td>5-4</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6-7</td>
<td>+ +</td>
<td>5-6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomycetes cerevisiae</td>
<td>5-5</td>
<td>+ +</td>
<td>5-0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pink torula</td>
<td>5-5</td>
<td>+ +</td>
<td>5-0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>5-5</td>
<td>++ +</td>
<td>5-0</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Controls were not gassed whereas the samples were pressurized to approximately 6 atm of either CO₂ or N₂. The pH of the CO₂ controls was the 'normal' pH of the medium, the pH of the CO₂ samples was the pH after the addition of the CO₂ and the pH for the N₂ controls and samples was adjusted to match that for the CO₂ samples. The degree of growth was defined as follows: + + +, luxuriant mould growth with aerial hyphae; + +, marked increase in colony count over initial count or moderate mould growth; +, definite growth or slight to moderate increase in colony count; ±, colony count remained approximately equal to the initial count; -, slight decrease in colony count from the initial count or no evidence of mould growth.

waters, distilled water and Berlin city water were used to demonstrate that carbonation removed the danger from cholera, but that there was still danger from typhoid fever, since *Salm. typhi* remained viable in carbonated waters for from 5 to 7 d (Hochstetter 1887), and in some cases growth continued unhindered (Fränkel 1889; Frankland 1896). *Vibrio cholerae*, *B. anthracis*, *Staphylococcus aureus*, *Staph. albus*, *Salm. typhi* and 'B. lepisepticum' were not affected by the concentration of CO₂ naturally dissolved (from the atmosphere) in water at 15°C, and only *V. cholerae* and *B. anthracis* were affected when the water was saturated with CO₂ (Scala & Sanfelice 1891). When *Pseudomonas fluorescens* var. liquefaciens, *Escherichia coli* var. communis, *Staph. aureus*, *Salm. typhi* and *V. cholerae* were inoculated into carbonated waters, killing or inhibition could be effected, the extent depending upon the organism and intensifying with time (Slater 1893). Reduction of pathogenic organisms, but not sterilization, could be obtained in carbonated beverages (Young & Sherwood 1911), the bactericidal action of CO₂ being assumed to be due to the increased hydrogen ion concentration of the solution (Koser & Skinner 1922; Valley & Rettger 1927). Later experimental data (Becker 1933; Hays et al. 1959), however, indicated that CO₂ *per se*, rather than its effect on pH, was the cause of the inhibitory effect (Table 1).

The decline in cell numbers of *E. coli* and *Salm. typhi* in artificially inoculated, carbonated beverages, was apparently proportional to the pressure and storage time (Donald et al. 1924), and apparent sterilization was achieved in only a few instances. When *E. coli* was subjected to 350 atm of CO₂ the number of bacteria was reduced by approximately one half in 20 min (Swearinger & Lewis 1933). More recently the aerobic growth rate of *E. coli* in fed-batch cultures was found not to be inhibited by 0-2 atm pCO₂, measured in the effluent gas, but 17% and 21% inhibition of growth rate was observed respectively at pCO₂ = 0.64 atm in complex medium and pCO₂ = 0.62 atm in defined medium (Mori et al. 1983). In the same study, inhibition of the growth rate of *Candida brassicae* was not observed until the pCO₂ in the effluent gas was 0.61 atm. During anaerobic growth of *E. coli* the optimum growth rate was at a dissolved CO₂ concentration of 1-3 mmol/l (=0.05 atm pCO₂ in the gas phase), above which the growth rate was inhibited (Lacoursiere et al. 1986). The perhaps not unexpected conclusion that was drawn from this, was that the maximum growth rate of *E. coli* occurs at CO₂ concentrations close to that found in the mammalian gut, where *E. coli* naturally resides.

The spoilage microflora of carbonated beverages are dominated by yeasts, and the carbonation tolerance of some of these was recently investigated (Ison & Gutteridge 1987). The most tolerant yeasts of those studied were *Dekkera anomala* and those of the genus *Brettanomyces*, a finding which
CO₂ inhibition

is in accordance with Brettanomyces spp. being the sole spoilage micro-organisms of soft drinks with high carbonation levels (Ison & Gutteridge 1987).

5. Treatment of dairy products with CO₂

The bacterial count of milk could be reduced by 50 atm of CO₂, but sterilization was still never obtained (Hoffman 1906). Milk kept under 10 atm of CO₂ remained in good condition for 72 h, whereas untreated milk curdled in 24 h. Increased pressures of CO₂ delayed lactic fermentation but no noticeable effect of CO₂ was observed at atmospheric pressure (Van Slyke & Bosworth 1907). Such findings led to a number of studies on the possible antibacterial effect of CO₂ in other dairy products.

Experiments on the effect of CO₂ on butter indicated that carbonation could not be relied upon as a means of destroying bacteria present in cream and rendering such cream, or the butter made from it, safe for human consumption (Hunziker 1924). Similarly, carbonation could not be relied upon to improve the keeping quality or to prevent flavour deterioration of the resulting butter. If it was made from unPasteurized cream, carbonated butter developed the usual bacterial flavour defects (Hunziker 1924). It was concluded that if any appreciable benefit was to be obtained from the carbonation of butter, it would be necessary to store the butter in a CO₂ atmosphere, although in time butter stored in this way also developed undesirable flavours (Prucha et al. 1925). Carbonation of the cream, or of the butter during curdling, did not result in any benefit to justify the use of CO₂ in this way (Prucha et al. 1925).

Carbonation of ice-cream had no appreciable effect on the bacteria within the ice-cream (Prucha et al. 1922; Rettger et al. 1922). It was demonstrated that CO₂ at atmospheric pressure had no bacteriostatic or bactericidal effect on organisms originally present in the ice-cream, or on Streptococcus lactis, E. coli or B. cereus added to it (Valley & Rettger 1927). No marked differences in viability could be discerned, and the slight variations favoured the aerated products and not the CO₂-treated products. In any event, after 7 weeks there was no difference in the CO₂ content of carbonated and uncarbonated ice-cream due to the ingress of the CO₂ by diffusion. Carbonation did not enhance the keeping quality of milk, nor prevent rapid bacterial proliferation of the pre-existing flora, nor of Strept. lactis, E. coli, Salm. typhi nor Salm. hirschfieldii inoculated into the milk (Valley & Rettger 1927).

Thus, although bacterial activities could be inhibited in the case of carbonated beverages, sterilization of water, milk or milk products was not possible simply by carbonation, and this was the general conclusion of a number of reports reviewed by Valley (1928). The carbonation of beverages was successful because the beverages were kept in air-tight containers under pressure. In order to inhibit the bacterial activities in dairy products higher pressures than those used in carbonated beverages, for which 4-8 atm were recommended (Donald et al. 1924), would be required (Prucha et al. 1925). The pH of carbonated beverages tends to be somewhat lower than that of milk, and since conditions causing inhibition are often synergistic, this would have also contributed to the success with carbonated beverages.

More recently, when milk was stirred under a headspace of 1 atm pCO₂ the pH fell to 6·0 and the CO₂ content increased to about 30 mmol/l. When this milk was subsequently held at 4 or 7°C there was a pronounced inhibitory effect on the growth of psychrotrophs, as compared with controls in air (Law & Mabbitt 1983). If 10⁶ bacteria/ml was taken to be the point when degradative changes in the milk become unacceptable, then the CO₂ treatment extended the possible storage time by about 3 d (at 4°C) or 2 d (at 7°C) for 'poor' quality milk and even longer for 'good' quality milk. The CO₂ could easily be removed before pasteurization, although this was not necessary if the milk was for cheese or yoghurt.

High concentrations of CO₂, combined with low oxygen concentrations, play an important role in the ripening of blue cheese. Penicillium roqueforti is mainly responsible for the ripening of blue cheese. As blue cheese ripens, its oxygen content decreases rapidly, and the CO₂ content rises. These conditions are unfavourable to all moulds, but they tend to affect Pen. roqueforti to a lesser extent than other species likely to be present and able to grow at the salt concentration found in blue cheese.
Partly in view of the growth-inhibitory effects of CO\textsubscript{2} that had been observed in some cases above, attempts, which began at approximately the same time as the early work on the carbonation of water, were also made to preserve meat and fruit in an atmosphere of CO\textsubscript{2}. Beef could be so preserved for 18 d in hot summer weather with daytime temperatures up to 32°C, whilst mutton showed spoilage in a very short time (Kolbe 1882). It is not known what the contribution of the pH was to this effect. Pork and lamb which went bad in 10 d in air at 4-5-7-2°C remained free from spoilage for 3 weeks when stored in CO\textsubscript{2} (Killeffer 1930). Partial pressures of CO\textsubscript{2} in the atmosphere suppressed mould growth on meat (Moran et al. 1932) and the growth of Pseudomonas and Achromobacter spp. in nutrient broth at 0 and 4°C (Haines 1933). Further work showed inhibition of the growth of such organisms on ox-muscle at $-1$°C in 0.1 atm pCO\textsubscript{2} (Empey & Scott 1939). The first practical use of modified atmospheres containing elevated levels of CO\textsubscript{2} as a preservative in the handling of fresh meat was in the shipment of whole chilled beef carcasses from Australia and New Zealand to Britain in the 1930s. By 1938, 26% of the beef from Australia and 60% of that from New Zealand was being shipped under CO\textsubscript{2} atmospheres (Lawrie 1974). The ability of high concentrations of CO\textsubscript{2} (>0.1 atm) to retard the growth of the Gram-negative spoilage flora of meat, poultry and fish, and in this way to prolong the shelf-life, is now well documented (Table 2) and modified atmosphere packaging has become the subject of over 4000 scientific papers in the past half decade (Lioutas 1988). Commercially the beneficial effects of CO\textsubscript{2} on the shelf-life of meat are used most during prolonged storage in refrigerated bulk containers (Taylor 1971; Dainty et al. 1983), as well as for wholesale and retail packages (Molin et al. 1983; Lioutas 1988; Young et al. 1988). Gas-packaging has also been applied commercially for poultry meat (Timmons 1976; Mead 1983) on an extensive scale in the US (Hotchkiss 1988). In addition to the use of CO\textsubscript{2} in gas-packaging, CO\textsubscript{2} pellets have been used to chill poultry (Thomson & Risse 1971), lamb carcasses and beef wholesale cuts during shipment from the packers to the retail trade (Smith et al. 1974).

### 6.1. INHIBITION OF THE GROWTH AND METABOLISM OF FOOD-SPOILAGE MICRO-ORGANISMS

In general, the rate of bacterial multiplication decreases, and the length of the lag phase increases, with increasing levels of CO\textsubscript{2} (e.g. Tomkins 1932; Haines 1933) and Gram-positive species are more resistant to the effects of CO\textsubscript{2} than are Gram-negative species (Sutherland et al. 1977; Silliker &

<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Clark &amp; Lentz 1972, 1973; Partman et al. 1975; Silliker et al. 1977; Sutherland et al. 1977; Christopher et al. 1979a</td>
</tr>
<tr>
<td>Pork</td>
<td>Huffman 1974; Silliker et al. 1977; Christopher et al. 1979b, 1980; Enfors et al. 1980</td>
</tr>
<tr>
<td>Cured meats</td>
<td></td>
</tr>
<tr>
<td>Smoked pork</td>
<td>Blickstad &amp; Molin 1983</td>
</tr>
<tr>
<td>Frankfurters</td>
<td>Ogilvy &amp; Ayres 1951b; Blickstad &amp; Molin 1983</td>
</tr>
<tr>
<td>Poultry</td>
<td>Ogilvy &amp; Ayres 1951a; Gardner et al. 1977; Sande &amp; Soo 1978; Mead 1983</td>
</tr>
<tr>
<td>Fish</td>
<td>Coyne 1932; Stansby &amp; Griffiths 1935; Banks et al. 1980</td>
</tr>
<tr>
<td>Cod</td>
<td>Coyne 1933a; Jensen et al. 1981</td>
</tr>
<tr>
<td>Herring</td>
<td>Molin et al. 1983b</td>
</tr>
<tr>
<td>Crayfish</td>
<td>Wang &amp; Brown 1983</td>
</tr>
<tr>
<td>Salmon</td>
<td>Ster et al. 1981; Fey &amp; Regenstein 1982</td>
</tr>
</tbody>
</table>

The use of CO\textsubscript{2} for the prevention of food-spoilage by micro-organisms and the possible mechanisms of action are discussed in the text.
$CO_2$ inhibition

Wolfe 1980; Stier et al. 1981). These effects vary with the concentration of $CO_2$, incubation temperature, organism and water activity of the medium (Wodzinski & Frazier 1961). Both aerobic respiration and growth rate were inhibited by $CO_2$ in fluorescent and non-fluorescent Pseudomonas spp., Alteromonas putrefaciens and Yersinia enterolitica, although the inhibition was incomplete (Gill & Tan 1980). The inhibition pattern was apparently biphasic for each organism, such that above a certain $pCO_2$ a further increase in $pCO_2$ had a minor inhibitory effect, as in an earlier report on Ps. fluorescens (Gill & Tan 1979). In contrast to such organisms, Acinetobacter (Gill & Tan 1980), Ps. aeruginosa (King & Nagel 1975), Ps. fragi, B. cereus and Strep. cremoris (Enfors & Molin 1980) all displayed a degree of inhibition by $CO_2$ that was more or less proportional to the $CO_2$ partial pressure over the entire $CO_2$ pressure range tested. The relative inhibitory effect of $CO_2$ (RI) was defined as:

$$RI = [(r_e - r_{CO_2})/r_e] \times 100$$  (10)

where $r_e =$ the growth rate of the control culture, and $r_{CO_2} =$ the growth rate of the $CO_2$-inhibited culture (Enfors & Molin 1981). As the temperature decreased, the RI for Ps. fragi and B. cereus increased (Enfors & Molin 1981), as was the case with most of the examples above. The ratio of the RI: $CO_2$ solubility at the corresponding temperature was approximately constant, and independent of temperature. Hence the increased inhibition by $CO_2$ at lower temperatures was explained as being due to increased $CO_2$ solubility, as opposed to increased susceptibility to $CO_2$. Since the initial microbial activity on muscle foods is located at the surface, however, it is a gas-solid interface that is being dealt with rather than a population dispersed in a liquid medium (Finne 1982a). The temperature effect may not therefore display the same characteristics for such an interfacial phenomenon as for a liquid medium. Results from a number of other authors were treated in a similar fashion: the RI: concentration of dissolved $CO_2$ was plotted against temperature and similar conclusions were drawn (Ogrydziak & Brown 1982), although in this case a number of the figures were plots of only two or three points.

The inhibitory effect of $CO_2$ on Pseudomonas spp. has been frequently studied (e.g. Clark & Lentz 1969 (several strains), King & Nagel 1975 (Ps. aeruginosa), Gill & Tan 1979 (Ps. fluorescens), 1980 (fluorescent and non-fluorescent strains), Enfors & Molin 1980 (Ps. fragi)) since this genus contains some of the major spoilage organisms of proteinaceous foodstuffs. Many of the results differ, however, with respect to the shape of the curve of growth rate versus $CO_2$ concentration. Explanations could be offered in terms of the effect of growth substrate or the inherent genetic differences between different species or strains. Some of the discrepancies may also be blamed on the application, in some cases, of fairly crude systems to measure the growth rate. All of the above experiments were carried out in batch cultures, where it was observed that the (maximum) specific growth rate of Ps. fragi decreased as the $pCO_2$ in the effluent gas was increased. However, results from continuous cultures revealed a shoulder (between 0.035 and 0.12 atm $CO_2$) in the curve of maximum specific growth rate (calculated from critical dilution rates) vs. $pCO_2$ that was not seen in the batch growth rate determinations (Molin 1983a). In oxygen-limited cultures the growth restricting effect of $CO_2$ was much moresevere (Molin 1983a).

Organisms which were $CO_2$-sensitive when grown aerobically were unaffected by $CO_2$ when grown anaerobically, as were Enterobacter spp. and Brochothrix thermosphacta (Gill & Tan 1980), suggesting that the sites of $CO_2$ inhibition during aerobic growth are different from those during anaerobic growth.

In general the partial pressures used in determining the degree of inhibition by $CO_2$ on the growth of the food-spoilage bacteria were between 0 and 1 atm. When hyperbaric $CO_2$ pressures were used it was found for instance that the time required to reach a given aerobic (sic) count was three times longer in 5 atm pure $CO_2$ than in 1 atm $CO_2$, and 15 times longer in 5 atm $CO_2$ than in air (Blickstad et al. 1981). These high partial pressures of $CO_2$ have a considerable effect in prolonging the shelf-life of unsterilized foodstuffs, by virtue of the fact that they select the microflora in favour of Lactobacillus spp. which are non-pathogenic (Shaw & Nichol 1969; Roth & Clark 1975) (these are the organisms that are among the least sensitive to high values of $pCO_2$ and by reducing the growth rate of even these Lactobacillus spp. (Ogilvy & Ayres 1953; Blickstad et al. 1981; Johnson et al. 1982;
Blickstad & Molin 1983). Hence the preserving action of CO₂ is not so much due to the control of the total microbial population as to a restriction of the types of organisms which most rapidly cause deterioration. The selection of the Lactobacillus spp. is viewed to have an added benefit, as many lactic bacteria are known to exert an antagonistic effect against other bacteria (Price & Lee 1970; Schroder et al. 1980). Nisin, of course, is well known as an antibiotic secreted by certain lactic acid bacteria (Fowler et al. 1975; Hurst 1981).

6.2. CO₂-RESISTANCE OF FOOD-RELATED BACTERIA

A number of attempts have been made to group different types of food-related bacteria with regard to their CO₂-resistance (e.g. Coyne 1933b; Sutherland et al. 1977; Gill & Tan 1980), but the methods used to evaluate growth rates were relatively crude. A more recent study (Molin 1983b) determined the resistance to CO₂ of a number of such food-related bacteria. Of the organisms studied, the relative growth-inhibitory effect of 1 atm pCO₂, as compared with the growth rate in air, was the highest (> 75%) for B. cereus, Brochothrix thermosphacta and Aeromonas hydrophila, and lowest (29–53%) for E. coli, Strep. faecalis and Lactobacillus spp. Under nitrogen the relative inhibitory effect of 1 atm pCO₂ on growth rate was lower than for aerobic growth; under anaerobic conditions it was highest for B. cereus, A. hydrophila and Y. frederiksenii (52–67%), and lowest for Y. enterolitica, Broch. thermosphacta and Lactobacillus spp. (8–26%). In 1 atm pCO₂ Strep. faecalis, Citrobacter freundii and E. coli had the highest maximum specific growth rates, and Broch. thermosphacta, B. cereus and Staph. aureus the lowest.

6.3. CO₂ IN THE PREVENTION OF FOOD SPOILAGE BY CLOSTRIDIA

Although 1 atm pCO₂ inhibited the germination of B. cereus spores, the same conditions stimulated the germination of spores of Clostridium sporogenes and Cl. perfringens (Enfors & Molin 1978a). Germination of Cl. sporogenes spores was inhibited slightly at 4 atm and almost completely at 10 atm, whereas germination of Cl. perfringens was slightly stimulated at 4 atm, unaffected at 10 atm and stopped at 25 atm of pure CO₂ (Enfors & Molin 1978a). In addition to decreasing the rate of spore germination, CO₂ has been shown to be lethal to certain clostridia (Hays et al. 1959). The number of spores of Cl. butyricum, Cl. botulinum and Cl. sporogenes are decreased after 42 d in the presence of 7-1 atm of CO₂.

Toxin production by Cl. botulinum was delayed in 1 atm pCO₂ when compared with that in 1 atm pN₂ at atmospheric pressure (Doyle 1983). Increasing the pressure of CO₂ further delayed the onset of toxin production, although production was not totally inhibited at 8:8 atm of CO₂. At high partial pressures, CO₂ was lethal to Cl. botulinum, the rate of decrease of colony-forming units being dependent both upon the CO₂ pressure and the length of exposure to it. However, 8 atm pCO₂ did not serve as a fully antibotulinial agent (Doyle 1983).

The effect of CO₂-enriched atmospheres on the behaviour of food-poisoning organisms in poultry products has received comparatively little attention. There was no significant difference between the effect of CO₂ and N₂ atmospheres, at 43°C, on the growth of eight strains of Cl. perfringens, although there was a slightly increased lag phase with two of the strains under CO₂ (Parekh & Solberg 1970). For one strain of Cl. perfringens incubated at 20°C the lag phase was 31 h under 1 atm pN₂ and 68 h under 1 atm pCO₂ (Mead 1983), which suggests that clostridial growth could at least be delayed by the presence of CO₂ under marginal growth-temperature conditions. In relation to chicken breast portions 1 atm pCO₂ markedly reduced the growth rate of the micro-organisms present (Gibbs & Patterson 1977).

6.4. PROBLEMS ASSOCIATED WITH THE PRESERVATION OF MEAT BY CO₂-CONTAINING ATMOSPHERES

The ability of modified atmospheres to preserve non-sterile foodstuffs has been shown to increase with increasing pCO₂. However, several workers indicate that the concentration of CO₂ which may
be used for meat storage is limited by the surface browning that occurs when the pCO₂ exceeds 0.2 atm (Pohja et al. 1967; Ledward 1970; Silliker et al. 1977), although others do not agree (Seidman et al. 1979). The browning was thought to result from metmyoglobin formation (Brown & Mebine 1969; Ledward 1970; Adams & Huffman 1972; Silliker et al. 1977) since surface browning does not occur in low-myoglobin-content foods, including seafoods (Coyne 1933a; Stansby & Griffiths 1935). This is of primary importance to the consumers who assume that an acceptable colour of the lean indicates freshness; for example the colour of pork chops changed from greyish pink to a less desirable brownish or tan colour as storage time increased (Adams & Huffman 1972). However, the odour of pork chops became objectionable before they were rejected because of appearance (Spahl et al. 1981)!

One way in which the development of the detrimental effect of CO₂ on the colour of meat may be circumvented by the addition of CO to the atmosphere, or by pretreatment with CO before the meat is placed in the CO₂-enriched atmosphere for storage (Silliker & Wolfe 1980). Because of safety considerations, however, CO in modified atmospheres has not yet been approved by the regulatory agencies for commercial use in the packaging of fresh foods (Finne 1982a). In most fresh red meat applications a mixture of gases is used and a number of combinations have been suggested e.g. 80% O₂/20% CO₂ (Georgala & Davidson 1970), 85–90% O₂/10–15% CO₂ (Clark & Lentz 1973), 75% CO₂/15% N₂/10% O₂ (Hotchkiss 1988). These gases and their concentrations should be tailored to the individual product, but for nearly all products this will be some combination of CO₂, N₂ and O₂. Nitrogen serves as a filler to keep the package from collapsing as CO₂ dissolves into the product (Hotchkiss 1988). Oxygen is used to maintain the bloomed colour of the meat (Young et al. 1988) and to inhibit the growth of anaerobic pathogens, but in many cases does not extend the shelf life (Hotchkiss 1988); CO₂ is added to inhibit the respiration of the product (Kadar 1980) in addition to the growth and metabolism of micro-organisms.

6.5. PRESERVATION OF SEAFOOD BY CO₂-CONTAINING ATMOSPHERES

Carbon dioxide has also been found to be effective in inhibiting the growth of the normal spoilage microbes of fish (Coyne 1932, 1933a, Finne 1982b; Parkin & Brown 1982; Tomlins et al. 1982), and modified atmosphere packaging, containing CO₂, is presently used in the seafood industry for bulk shipments (Banks et al. 1980; Bell 1980; Beals 1982; Wilhelm 1982). CO₂-enriched atmospheres have also been effective in retarding microbial growth during refrigerated storage of retail packaged seafood products (Finne 1982c; Lannelongue et al. 1982a).

As most of our seafoods are still harvested from the wild, the microbial flora is usually diverse, with the majority belonging to the genera Pseudomonas, Acinetobacter, Moraxella, Flavobacterium-Cytophaga and Arthrobacter, and will of course be affected by the environment from which the seafood is harvested (i.e. regional and seasonal differences), physiological condition of the animal, time between harvest and arrival at the docks, onboard handling, etc. (Lee 1982).

Concern has been expressed, in terms of controlled and modified-atmosphere storage (Clark & Takacs 1980), with regard to the psychrotrophic Y. enterolitica, and Campylobacter fetus ss. jejuni, since CO₂-enrichment is in fact used for selective isolation of these organisms (Skirrow 1977). However, seafood has not been implicated in infections caused by these organisms (Lee 1982).

Further concern with modified atmosphere storage of seafoods was related to the possibility of toxin production by Cl. botulinum, especially the psychrotrophic type E, before the fish spoils (Stier et al. 1981; Eklund 1982a, 1982b; Post et al. 1983), as up to 0.9 atm CO₂ had no effect on growth or toxin production (Silliker & Wolfe 1980, Lee 1982). To overcome this problem, fish could be stored refrigerated in CO₂ up to the period of retail display and then transferred to air (Bell 1982). For pork and beef there was a residual inhibitory effect of the CO₂-containing atmosphere after transfer to air (e.g. Silliker et al. 1977; Enfors et al. 1979; Silliker & Wolfe 1980; Wolfe 1980; Spahl et al. 1981), an effect that was also observed with poultry (Ogilvy & Ayres 1951a; Bailey et al. 1979; Silliker & Wolfe 1980).

While it may not be possible to prove that the potential contamination of fish, by Cl. botulinum toxin, stored in CO₂-rich atmospheres does not exist, there is evidence that it may not present a significant risk provided proper sanitation and temperature controls are employed (Daniels et al.
1985). The potential for contamination could also be reduced by maintaining the temperature below 3-3°C (Schmidt et al. 1961). Clostridial growth and toxin production has been detected in fish stored in vacuum- and CO₂-packages, but, in all instances, the fish was spoiled beyond hope of human consumption (Banner 1978). Until safety from botulism can be demonstrated, the use of packaging involving low oxygen concentrations cannot be recommended for retail use (Wilhelm 1982). However, even if the toxin is formed, normal cooking operations will inactivate it (Licciardello et al. 1967).

Although CO₂ was effective in prolonging the shelf-life of fresh fish (the most effective combinations of gas were 1 atm CO₂ or 0-4 atm CO₂/0-6 atm N₂ (Lannelongue et al. 1982b)), the rate of microbial growth in the fish after the removal of the CO₂ paralleled the rate of growth in fish stored without CO₂ (Banks et al. 1980). A residual inhibitory effect on microbial growth due to storage in 0-8 atm CO₂ was demonstrated for cod fillets stored in a modified atmosphere and then transferred to air at 4°C (Wang & Ogrydziak 1986). It was suggested that this residual effect was not due to retention of CO₂ at the surface of the fillets but was probably due to the microbial ecology of the system. After 7 d storage, with CO₂, Lactobacillus spp. and Alteromonas spp. were predominant, and 6 d after transfer to air Pseudomonas spp. were again dominant (Wang & Ogrydziak 1986).

Pseudomonads, which are among the major spoilage organisms for seafood stored refrigerated in air (Shaw & Shewan 1968), were inhibited during storage in CO₂ (Enfors & Molin 1980; Gill & Tan 1979). One possible problem during the storage of non-sterile foodstuffs in elevated CO₂ atmospheres is that the spoilage flora may genetically adapt to high levels of CO₂, as appeared to be the case with Pseudomonas spp.-like strains isolated from rock cod (Johnson & Ogrydziak 1984). On rock cod fillets stored in 0-8 atm CO₂ at 4°C, Lactobacillus spp. and an Aeromonas-like organism became the predominant organisms, whilst V. paraohaemolyticus, Staph. aureus or Cl. botulinum type E were not isolated from fresh or modified atmosphere stored fillets (Mokhele et al. 1983). Similar results were obtained with herring fillets (Molin et al. 1983). The initial microflora of fresh herring was dominated by coryneforms, Flavobacterium spp., Moraxella-like organisms and Pseudomonas spp. In air the spoilage flora was dominated by Pseudomonas spp. and Moraxella-like organisms, whereas homofermentative Lactobacillus spp. were the only organisms isolated from herring fillets stored in 1 atm CO₂ at 2°C (Molin et al. 1983).

In addition to retarding microbial growth on seafoods, CO₂-enriched atmospheres are also effective in reducing the rate of amine production within the seafood itself (Brown et al. 1980, Parkin et al. 1981; Johnson et al. 1982; Watts & Brown 1982). Whether this is a direct effect on the redox activities of the components of the respiratory chain leading to trimethylamine N-oxide is not apparently known.

7. Preservation of fruits and vegetables by CO₂

Much of the value of CO₂ treatment of fruits is due to the delay of their rotting by fungi but this is not completely attributable to a direct influence on the fungal organisms, since pCO₂ influences the physiological conditions of the host tissue itself (Smith 1963). CO₂ is not in general as important a factor in reducing the amount of fungal growth as is lowering the temperature (Brown 1922). Partial pressures of CO₂ in the range of 0-2-0-5 atm nonetheless provided a strong check to fungal growth at all temperatures (Brown 1922; Brooks et al. 1932). In comparison with air, 1 atm CO plus 0-5 atm CO₂, plus 0-23 atm O₂ gave an 80-90% reduction of rot development in strawberries (from Botrytis cinerea), apples (Pen. expansum), lemons (Whetzelinia sclerotiorum) and oranges (Pen. italicum and Pen. digitatum) (El-Goorani & Sommer 1979). The effectiveness of the CO₂ treatment depends, of course, upon the amount of nutrient available to the fungus, and with greater retardation of fungal growth (due to CO₂) at lower temperatures (Brown 1922; Moran et al. 1932). The early literature concerning the effects of CO₂, including the prevention of fungal growth, on the storage of a variety of fruits and vegetables has been reviewed by Smith (1963). The inhibitory effects of CO₂ have also been exploited in the Böhi process for the preservation of grape juice (Jenny 1952). More recently the use of modified atmospheres, containing CO₂, have been limited to the international movement of selected vegetables and fruits and for large-scale domestic transport of apples, pears, citrus fruits and

8. Control of the production of fermented foods and beverages using CO₂

It has been indicated above that under appropriate conditions CO₂ can preserve foods by virtue of its inhibitory influence upon certain food-spoilage organisms. However, the production of many foodstuffs of course actually relies upon fermentation processes (Haas 1976, Rose 1981, 1982; Erichsen 1983; Marshall & Law 1984; Campbell-Platt 1987). Thus, CO₂ may also be used as a means by which to control or affect the properties of foods produced by fermentation. An example of this is the use of exogenous CO₂ as a controlling agent in the production of alcoholic beverages by the brewing industry (Hoggan 1980). The final concentration of many flavour compounds and esters may be decreased by increasing the CO₂ pressure during fermentation (Drost 1977). The fermentation rate, the rate and extent of yeast growth, and the final concentration of fusel oils are all decreased by increasing CO₂ pressure, whilst the final pH is increased (Kunkee & Ough 1966, Jones & Greenfield 1982; Arcay-Ledezma & Slaughter 1984).

Fermentation of malt extract medium by Saccharomyces cerevisiae under a CO₂ pressure of 1.97 atm resulted in a changed pattern of absorption of amino acids in the first 4 h, with a general excretion of amino acids thereafter (Slaughter et al. 1987). Growth of Sacch. cerevisiae was almost completely stopped by 2.7 atm of CO₂, but no changes in growth rate were observed in 2.7 atm of N₂ (Norton & Krauss 1972). At much higher pressures, of some 41 atm, inhibition of the growth rate of many organisms occurs, as demonstrated with Strep. faecalis at high hydrostatic pressures and high partial pressure of inert gases, including nitrogen (Fenn & Marquis 1968). Inhibition of cell division and of the production of new buds by Sacch. cerevisiae was caused by CO₂ when it is produced endogenously or added. In contrast, metabolic production of CO₂ was unaffected by endogenously-produced pressures which inhibited the cell division of this organism. Although cell division was inhibited, doubling of the DNA content of the cells still occurred, indicating that the inhibition of cell division was not due to the inhibition of DNA replication (Norton & Krauss 1972). Despite the increase in DNA content of the cells the content of RNA and protein per cell decreased (Lumsden et al. 1987). After one hour at elevated CO₂ pressures the mean cell volume of Sacch. cerevisiae had increased, whereas the mean cell volume of Schizosaccharomyces pombe had decreased, suggesting that the influence of CO₂ upon cell characteristics may be associated with a change in cell volume (Lumsden et al. 1987). The effects of CO₂ on Schiz. pombe were otherwise the same as the effects of CO₂ on Sacch. cerevisiae. The inhibition of cell division by CO₂ has also been observed in algae. After 8 h in 5% CO₂ in air Chlorella spp. did not reveal any internal subdivision into daughter cells, despite being shown to be capable of division (Sorokin 1962).

9. Inhibition of other industrial fermentation processes by CO₂

The use of CO₂ for the control of fermentation end-products is not restricted to the brewing industry, and indeed there are many examples in which CO₂ can affect the progress of industrial fermentations (Table 3), for good or for ill. CO₂ pressures may significantly influence the regulation of microbial metabolism favouring biomass or product formation (Mudgett & Bajracharya 1979; Bajracharya & Mudgett 1980).

9.1. Effects of CO₂ on the Production of Biomass

The partial pressure of CO₂ may be increased to several atmospheres in some fermenters, but in some cases, for example pressure-cycle fermenters such as the 'Pruteen' plant at Billingham, UK, the partial pressure of CO₂ may fluctuate over a substantial range. In the case of the 'Pruteen' plant the pCO₂ may change from some 0.45 atm to 0.05 atm in 1 min (Vasey & Powell 1984); the CO₂ concentration
is at its highest close to the base of the riser, where growth is the greatest. Growth of the organism used for the 'Pruteen' process, *Methylophilus methylotrophus*, was found to be sensitive to CO$_2$, with the maximum specific growth rate decreasing from 0.5 to 0.15/h as the partial pressure of CO$_2$ increased from 0.05 to 0.4 atm. Above 0.29 atm CO$_2$ the product specification changed and the carbon-to-cell conversion decreased (Vasey & Powell 1984).

During biomass production with bakers' yeast, inhibition of the extent of yeast growth below 0.2 atm pCO$_2$ in the gas phase was negligible, with slight inhibition at 0.4 atm and significant inhibition at 0.5 pCO$_2$ (Chen & Gutmanis 1976). In the case of ethanol production by *Zymomonas mobilis* biomass production is inhibited by CO$_2$ (Schreder *et al.* 1934) and it was noted that nucleation, by adding diatomaceous earth, or additional stripping of CO$_2$, increased the glucose uptake rate during the early stages of fed-batch fermentation (Burrill *et al.* 1983). During continuous cultivation, at high dilution rates, biomass production was increased by as much as 100% when CO$_2$ was decreased from 1.46 atm to 0.095 atm (Nipkow *et al.* 1985). Product yield was not affected by CO$_2$ partial pressure, but the glucose uptake and ethanol production rates decreased as the partial pressure of CO$_2$ was decreased. Nitrogen sparging was found to reduce lag times considerably in batch cultures, probably because of the removal of CO$_2$ (Veeramallu & Agrawal 1986). In this study, however, no noticeable trend in glucose uptake or ethanol production rates were observed when CO$_2$ was removed from the culture medium. Despite this the specific growth rate increased by 15% and the cell mass yield increased by 12%, but the overall ethanol yield decreased by 5%. Production of CO$_2$ was shown to be directly coupled with ethanol formation but not necessarily with cell mass production, indicating a decoupling of growth from ethanol production (Veeramallu & Agrawal 1986). It was noted that high pCO$_2$ combined with high ethanol concentrations caused a change in the morphology of *Z. mobilis*, leading to the appearance of extensive slime and granular layers around the cells (Doelle & McGregor 1983). Alteration of the cellular morphology has also been obtained with *Strep. mutans*, the shape of which could be dictated by the ratio of CO$_2$ in the form of bicarbonate, to K$^+$ in the growth medium (Tao *et al.* 1987). A high bicarbonate/K$^+$ ratio produced spherical cells, whereas the cells remained bacillary in medium with a low bicarbonate/K$^+$ ratio.

---

**Table 3. A summary of some of the industrial fermentation processes that are inhibited by CO$_2$.**

<table>
<thead>
<tr>
<th>Product/process inhibited by CO$_2$</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass 'Pruteen'</td>
<td>Vasey &amp; Powell 1984</td>
</tr>
<tr>
<td>Baker's yeast</td>
<td>Chen &amp; Gutmanis 1976</td>
</tr>
<tr>
<td>Solvents</td>
<td>Klei <em>et al.</em> 1984</td>
</tr>
<tr>
<td>Amino acids</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>Akashi <em>et al.</em> 1979; Hirose 1986</td>
</tr>
<tr>
<td>Histidine</td>
<td>Akashi <em>et al.</em> 1979; Hirose 1986</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Hirose <em>et al.</em> 1968</td>
</tr>
<tr>
<td>Inosine</td>
<td>Shibai <em>et al.</em> 1973; Ishizaki <em>et al.</em> 1973</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>Ho &amp; Smith 1986</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Tikhonov <em>et al.</em> 1983</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td>Bylinkina <em>et al.</em> 1973</td>
</tr>
<tr>
<td>Streptomyccin</td>
<td>Bylinkina <em>et al.</em> 1973</td>
</tr>
<tr>
<td>Methane</td>
<td>Hansson 1979</td>
</tr>
<tr>
<td>Degradation of lignocellulosic wastes</td>
<td>Drew &amp; Kadam 1979; Mudgett &amp; Paradis 1985</td>
</tr>
</tbody>
</table>

These processes are discussed in detail in the text of section 9.
9.2. EFFECTS OF CO₂ ON THE PRODUCTION OF BIOCHEMICALS

During the production of acetone and butanol by Cl. acetobutylicum, the butanol/acetone ratio is significantly changed at CO₂ pressures above 2.6 atm, with the virtual elimination of ethanol production (Klei et al. 1984). Maximum solvent production by Cl. acetobutylicum, at the expense of cell growth, was found to occur at 1.7 atm and substrate utilization was inhibited with increasing pCO₂. The ability to shift the product distribution would allow the fermentation plant manager to respond to changes in demand and price. In contrast to the effects of CO₂ on the product ratios of Cl. acetobutylicum, the partial pressure of CO₂ does not noticeably influence the fermentation products of Cl. butyricum (van Andel et al. 1985).

During aerobic batch growth of Brevibacterium flavum on glucose under controlled gas phases, CO₂ was found to affect amino acid production (Akashi et al. 1979; Hirose 1986). At all pCO₂ values CO₂ reduced the yield of histidine, produced by Brev. flavum, whilst arginine displayed an optimal yield at a pCO₂ of 0.12 atm, above which the yield decreased rapidly. In this instance, as in the case of the fermentation by Cl. acetobutylicum, it is thus possible to control the ratio of products by the use of CO₂.

During glutamic acid production by Brev. lactofermentum high partial pressures of CO₂ caused a decrease in glutamate production, sugar consumption and respiratory activity (Hirose et al. 1968). High CO₂ tension also resulted in a decrease in product formation in a nucleoside fermentation employing a mutant of B. subtilis (Shibai et al. 1973). In the same fermentation, the yield of inosine was shown to be independent of the bicarbonate ion concentration in the culture medium, but greatly reduced by increasing partial pressures of CO₂ (Ishizaki et al. 1973).

The production of antibiotics is inhibited by relatively low partial pressures of CO₂. The rate of synthesis of penicillin by Pen. chrysogenum was halved by a CO₂ partial pressure of 0.08 atm (Pirt & Mancini 1975), when compared with the rate of synthesis at 0.006-0.007 atm pCO₂. Exposure to influent gases of 0.03 and 0.05 atm of CO₂ produced no observable inhibition of the metabolism of Pen. chrysogenum, but influent gases of 0.126 and 0.2 atm of CO₂ inhibited both growth rate and penicillin production rate (Ho & Smith 1986). Tetracycline biosynthesis was found to be optimal at 0.02 atm CO₂ (Tikhonov et al. 1983) and the inhibition by CO₂ of the production of other antibiotics, such as oleandomycin and streptomycin, have also been reported (Bylinkina et al. 1973).

9.3. EFFECTS OF CO₂ ON THE TREATMENT OF WASTES

Any inhibitory effect of CO₂ could be of great importance in methane production during the digestion of wastes, since the partial pressure of CO₂ could increase to several atmospheres in large digestors. In general, rapid increases in pCO₂ resulted in rapid decreases in methane production and vice versa (Hansson 1979). The methane yields were 20-30% higher at low values of pCO₂ compared with those at 1 atm CO₂ (Hansson & Molin 1981). Combined with decreasing methane yields, as pCO₂ increases from 0 to 1 atm, there is a progressive inhibition of acetate and propionate degradation, although some CO₂ was required for propionate degradation since the rate of degradation dropped rapidly below 0.2 atm CO₂ (Hansson & Molin 1981; Hansson 1982). High pCO₂ also decreased the temperature maximum at which bacterial acetate degradation occurred (Hansson 1982).

Conversion of lignocellulosic wastes is also susceptible to inhibition by CO₂; increasing CO₂ pressure increasingly inhibits the rate and extent of degradation of lignin and non-lignin materials by Phanerochaete chrysosporium (Mudgett & Paradis 1985). Lignin degradation rates were also suppressed by high levels of CO₂ in the later stages of the fermentation catalysed by Aspergillus fumigatus (Drew & Kadam 1979). Both inhibitory and stimulatory effects of CO₂ have been reported for a number of other fermentations (Mudgett 1980). It may be concluded therefore that in some cases CO₂ exerts a controlling influence upon microbial growth and metabolism which may be utilised for biotechnological purposes. It is therefore germane to enquire into some of the possible mechanisms whereby CO₂ exerts its inhibitory effects at the subcellular level.
10. Sites of action

10.1. BIOLOGICAL MEMBRANES

As it has been noted above that CO₂ may inhibit cell division and cause alterations in cell morphology, glucose uptake rates and amino acid absorption, it would be reasonable to suggest that such effects of CO₂ may be associated with changes in the function of the biological membrane. Indeed one of the factors implicated in contributing to the reason for the growth-inhibitory effects of CO₂ has been the alteration of membrane properties (Sears & Eisenberg 1961). It was suggested that CO₂ interacts with lipids of the cell membrane, decreasing the ability of the cell to uptake various ions. ‘Anaesthesia’ (i.e. narcosis) caused by elevated levels of pCO₂ was proposed as a basis for the effect of CO₂ on the membrane during CO₂-mediated growth inhibition, and the available literature on yeast was reviewed (Jones & Greenfield 1982). Changes in the permeability of the cell membrane were also invoked in connection with a study of the effects of CO₂ on the germination of bacterial spores (Enfors & Molin 1978b). The inhibition was suggested to be due to an increase in fluidity, causing the disturbance of the activity of a membrane-bound enzyme essential to the initiation of germination. Alterations in the fatty acid content, and fluidity, of the yeast cell membrane at elevated pCO₂ levels were noted (Castelli et al. 1969).

One theory of narcosis is that absorption of an ‘anaesthetic agent’ into a biological membrane causes a hydrophobic region to expand beyond a certain critical size. This theory is termed the critical volume hypothesis (Miller et al. 1973) and is consistent with the observations that anaesthetics expand monolayers, bilayers and bulk solvents (Seeman 1972) and that hydrostatic pressure can in some cases reverse anaesthesia (Lever et al. 1971). Expansion of biological membranes at clinically significant concentrations of general anaesthetic is estimated to be of the order of 0.4% (Lever et al. 1971). It has been demonstrated that anaesthetics disorder the lipid bilayer of the membrane (Jain et al. 1975, Vanderkooi et al. 1977, Pang et al. 1980). Perturbations in membrane fluidity, caused by the disordering of the lipid bilayer, are postulated to alter the function of membrane proteins, resulting in the changes that may be associated with anaesthesia (Chin et al. 1976; Roth 1980). The phase transition hypothesis (Lee 1976) suggests that lipids surrounding functional membrane proteins exist in a gel phase (as a lipid annulus) retaining proteins that form ion channels in an open state, and anaesthetics ‘melt’ this rigid lipid (Roth 1980). Regions in which lipids in the liquid phase coexist with lipids in the gel phase are termed lateral phase separations (Trudell 1977) and are associated with functional changes in membrane proteins, possibly modulating the protein conformation through hydrophobic or electrostatic interactions. Anaesthetics may reduce these lateral phase separations, thus altering the functional activity of the proteins (Roth 1980).

In each of the above theories the anaesthetic is taken to be interacting with the lipid portion of the membrane, and these theories are known as lipid theories of narcosis. In contrast with these theories are the protein theories of narcosis. These latter theories proposed that narcosis is due to a direct effect of the anaesthetic upon membrane proteins (Richards et al. 1978; Franks & Lieb 1986). The degenerate perturbation hypothesis (Richards et al. 1978) proposes that small molecules are distributed in one set of hydrophobic sites of appropriate dimensions within membrane proteins, and larger molecules may bind to distinct sets of sites on the membrane proteins. In addition to this, molecules with structural features similar to those of phospholipids will compete for the regions of the proteins that interact with phospholipids; the replacement of annular lipids by anaesthetic molecules then interferes with the function of the protein.

n-Alcohols behave as anaesthetics; their potency increases until a certain chain length is reached, beyond which their biological activity ceases. The lipid theories state that this ‘cut-off’ in anaesthetic activity is due to a corresponding cut-off in the absorption of the alcohols (that are then longer than the specific chain length) into the lipid bilayer of the membrane. However, it has been demonstrated that the partition of alcohols into lipid bilayers continues long after their biological activity has ceased (Franks & Lieb 1986). This supports the view that it is the membrane proteins and not the lipid bilayers, that are the target sites for anaesthetics. The general anaesthetic properties of the n-alcohols can be accounted for in terms of binding to protein target sites of circumscribed dimen-
sions: the levelling off in potency occurs when the site becomes full, and the cut-off point occurs when the concentration for anaesthetic activity falls below the aqueous solubility (Franks & Lieb 1986).

Narcosis may also be caused by the action of CO$_2$ on the cytoplasm as well as on the plasma membrane. During CO$_2$ narcosis of the filamentous alga, *Nitella clavata*, there is a gradual reversible sol-to-gel conversion of the cytoplasm, assumed to be associated with the carbamination of proteins (Fox 1981). Removal of CO$_2$ from the gas phase restores the sol state of the cytoplasm from its partially gelled condition. Also involved in the narcosis of the cytoplasm is the exosmosis of water and electrolytes. The carbamination and subsequent coalescence of enzymic proteins would presumably deprive them of their full function as catalysts (Fox 1981).

Reaction of amino groups, present on membrane proteins, with CO$_2$ will result in potentially positive ions (RNH$_3^+$) becoming potentially negative ions (RNHCOO$^-$) (Mitz 1979). This charge change causes a change in the surface potential and may selectively favour the transport of positive ions, whilst inhibiting the transport of negative ions across the membrane. In addition to this there would probably be a significant conformational change associated with the charge change, which would also affect the function of the protein.

The inhibition by CO$_2$ of the uptake of amino acids by membrane vesicles of *E. coli* and *B. subtilis* was only one half that of the inhibition of growth, whereas the uptake of glucose by membrane vesicles of *E. coli* was unaffected by 100% CO$_2$ (Eklund 1984). This might be interpreted to mean that the main mechanism of CO$_2$ action is not associated with the biological membrane. Such an interpretation can now be seen to be incorrect when viewed in terms of the so-called metabolic control analysis (e.g. Kacser & Burns 1973; Westerhoff *et al.* 1984), which is discussed in the next section.

### 10.1.1. The metabolic control analysis

The metabolic control analysis was originally formulated to describe the control of metabolic pathways. When considering the control of metabolic pathways it is traditional to ask the question 'which step is rate limiting?', when referring to pathway substrates or products. However, the question that should more properly be asked of a parameter is 'how rate limiting is it?', and it has become apparent that the appropriate formalism with which quantitatively to approach such a question is the metabolic control theory (see e.g. Kacser & Burns 1973; Kell & Westerhoff 1986a, b, Kacser & Porteus 1987; Westerhoff & Kell 1987, Kell *et al.* 1989). This theory derives from the general formalism of sensitivity analysis (Cruz 1973), in which the sensitivity of any variable to any parameter is expressed as a 'sensitivity coefficient' or a 'control coefficient'. The control coefficient of an enzyme, which expresses in quantitative terms the degree to which it may be rate limiting to the flux through a metabolic pathway, is defined (e.g. Kell & Westerhoff 1986b) as

$$C_i' = [(dJ/d\varepsilon_i) \times (\varepsilon_i/J)]_s^s$$

$$= (d \log |J|/d \log \varepsilon_i)_s$$

where $C_i'$ is the flux control coefficient of enzyme $i$, $J$ is the flux through the pathway, $\varepsilon_i$ is the concentration of enzyme $i$ and $ss$ denotes steady state. It should be noted that the differentials apply strictly to infinitesimal changes in the parameter and the variable studied, but, particularly to accommodate values of the parameter equal to zero, it is permissible to use the (small but finite) values of the changes themselves (i.e. $\delta J, \delta \varepsilon_i$) to calculate the control coefficients. The control analysis contains a number of other theorems relating system properties such as fluxes to the properties (the so-called elasticities) of individual enzymes. The metabolic control theory can be applied to processes other than flux through metabolic pathways, as in our work on the effects of CO$_2$ upon the growth rate of *Cl. sporogenes* (Dixon *et al.* 1987). Hence the above statement, that the main mechanism of CO$_2$ action is not associated with the biological membrane, may be incorrect, as this mechanism will contribute to the overall effect of CO$_2$ on the cell. If the control coefficient on growth for a membranous enzyme (times the elasticity of the same enzyme towards CO$_2$) is greater than that for any other similar interaction, then it will in fact be the 'main' mechanism of action. Further, the value of the
control coefficient will tend to vary with the environmental conditions, so in one set of conditions one enzyme may exert the most control, but in another set of conditions it may be another enzyme that exerts the most control on the growth rate.

10.2. CYTOPLASMIC ENZYMES AS A SITE OF ACTION OF CO₂

Rather than a generalized effect on membrane-located proteins, CO₂ may exert its influence upon a cell by affecting the rate at which particular reactions proceed. One way in which this may be brought about is of course to cause an alteration in the production of a specific enzyme or enzymes, via induction or repression of enzyme synthesis (Jones & Greenfield 1982; Sarles & Tabita 1983; Bowien & Leadbeater 1984; Dixon 1988). It was suggested (Wimpenny 1969), and evidence has since been obtained (Wood & Stjernholm 1962; Wood & Utter 1965; Wimpenny 1969; Jones & Greenfield 1982), that the primary sites at which CO₂ exerts its effects are the enzymatic carboxylation and decarboxylation reactions, although the effects extend to enzymes not necessarily involved in carboxylation or decarboxylation reactions (Jones & Greenfield 1982).

There have been a number of studies showing that CO₂ causes alterations in the rate of certain enzymatic reactions in bacteria, leading to the build-up of the concentrations of certain metabolites. Thus, increasing the concentration of CO₂ caused the rate of succinate formation by _E. coli_ var. _commune_ to increase, the removal of CO₂ causing a decrease in the succinate concentration (Elsden 1938). pCO₂ values of 0·01 to 0·1 atm affected the enzymes of _Sclerotium rolfsii_, causing a decrease in the succinate dehydrogenase activity and significant increases in the isocitrate lyase, isocitrate dehydrogenase, malate synthase and malate dehydrogenase activities, compared to when the organism was grown in air (Kritzman et al. 1977).

10.2.1. CO₂-controlled induction/repression of enzyme synthesis

The control exerted upon the enzymes of _Sclerotium rolfsii_, as mentioned above, was through induction/repression of enzyme synthesis. Similarly the control of autotrophic CO₂ fixation by CO₂ is mostly by induction or repression of enzyme synthesis. When CO₂ was supplied at low partial pressures (= 0·02 atm in hydrogen) to photolithotrophically grown cells of _Rhodospirillum rubrum_, up to 50% of the soluble protein was ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBPCase); increasing the pCO₂ from 0·02 to 0·05 atm resulted in a rapid and dramatic decrease in the rate and extent of RuBP synthesis. Lowering the CO₂ back to 0·02 atm resulted in a dramatic increase in RuBPCase synthesis (Sarles & Tabita 1983; Tabita et al. 1985). This response to the partial pressure of CO₂ appeared to be typical of other chemotrophic and phototrophic organisms, although they may not be as sensitive to CO₂ or synthesize the same amount of RuBPCase as _Rhodo. rubrum_ (Sarles & Tabita 1983; Tabita et al. 1983, 1984). CO₂ limitation of _Alcaligenes eutrophus_ depresses the synthesis of RuBPCase and phosphoribulokinase (PRK) to about one-fifth of the normal autotrophic level (Friedrich 1982; Bowien & Leadbeater 1984). RuBPCase is activated by CO₂ (Lorimer 1981a; Miziorko & Lorimer 1983). The reversible activation process is accompanied by a carbamate formation at a specific activator site lysine on the large subunit of the enzyme (Lorimer & Miziorko 1980; Lorimer 1981b). Activation of RuBPCase from _Alc. eutrophus_ was shown to result in a significant change of the hydrodynamic properties of the enzyme. The inactive enzyme exhibited a sedimentation coefficient, _S_{20,w},_ of 17·5 S, whereas activation lowered this value to 14·3 S (Bowien & Gottschalk 1982). This alteration reflects major changes in the tertiary structure of the enzyme, in which the small subunits could have a crucial function. For further information about the control of CO₂ fixation see also Tabita (1981), Dijkhuizen & Harder (1984) and Bowien et al. (1987).

10.2.2. CO₂ inhibition of enzyme reactions

Another way in which CO₂ may influence the rate of a reaction is to inhibit or stimulate the reaction, rather than affecting the synthesis of the appropriate enzyme. Formate hydrogenlyase of _E. coli_ was also inhibited by CO₂ (Swanson & Ogg 1969). Fumarate formation by _Rhizopus nigricans_ was
CO₂ inhibition

blocked at the pyruvate carboxylation step due to the inhibition of oxaloacetate decarboxylase activity by CO₂ (Foster & Davis 1949). Both malate dehydrogenase and isocitrate dehydrogenase of Ps. aeruginosa were found to be inhibited by CO₂, but oxaloacetate decarboxylase, fumarase, succinate dehydrogenase and cytochrome c oxidase were unaffected, although a small amount of inhibition might have been undetected (King & Nagel 1975). Catalase activity was reversibly decreased by half in 0.1 atm pCO₂ (Bretskein & Ivanova 1955), although the peroxidatic activity of the enzyme was enhanced (Mitsuda et al. 1958). The extracted protease activity of Ps. fragi was inhibited by increasing concentrations of CO₂, whereas in the case of Streptomyces caespitosus the extracted protease activity exhibited an optimum pCO₂ of 0.4 atm pCO₂ in argon as compared with its activity in pure argon (Pichard et al. 1984).

11. Mechanisms of CO₂ inhibition of microbial growth and metabolism

Despite numerous reports of the effects of CO₂ on microbial growth and metabolism, a number of which have been discussed, the ‘mechanism’ of CO₂ inhibition still remains unclear. From what has been written above, a unitary mechanism of CO₂ inhibition seems out of the question. However, a number of possible explanations have been postulated.

In an anaerobic chemostat culture of Klebsiella aerogenes it was demonstrated that increased concentrations of metabolically produced CO₂ effected a lowering of yield values (Teixeira de Mattos et al. 1984). CO₂ exerts an effect on both metabolism and the energetics of cell synthesis. In this case the proposed mechanism was one of a futile cycle, dissipating energy, stimulated by increased CO₂ concentrations and involving carboxylation and decarboxylation reactions, the net result being ATP → ADP + P_i (Teixeira de Mattos et al. 1984 and see Fig. 1a). Such effects of CO₂ on growth energetics have been observed with other organisms. During the growth of Cl. sporogenes in ‘glucose-limited’ chemostat culture in a defined minimal medium in which this organism required CO₂ for growth (Dixon et al. 1987; Lovitt et al. 1987), CO₂ induced some type of metabolic ‘slip’, since both the yield coefficient and the apparent maintenance requirements decreased as the pCO₂ was increased above the optimal pCO₂ (0.5 atm) for growth rate in ‘unrestricted’ batch culture (Dixon et al. 1988; see also Pennock & Tempest 1988 for similar energetic behaviour in B. stearothermophilus). A futile cycle stimulated by CO₂ and pH, involving the CO₂/HCO₃⁻ equilibrium was proposed as a possible mechanism of energy dissipation in the former case (Fig. 1b). The occurrence of futile cycles will have an increasing importance, to the cell, as the growth rate is decreased e.g. in nature.

A non-equilibrium thermodynamic assessment of the ‘efficiency of growth’ of heterotrophic bacteria indicated that they have in general evolved to permit a maximum metabolic flux of the carbon and energy-source at the expense of efficiency or yield, so that the thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate (Westerhoff et al. 1983; Kell 1987). When Cl. sporogenes was grown in chemostat culture in a medium in which CO₂ was not required for growth (Dixon et al. 1987; Lovitt et al. 1987), as the pCO₂ was increased above the optimal pCO₂ for growth rate in ‘unrestricted’ batch culture, the thermodynamic efficiency of growth was increased (in that both yield (Y_{x/μ}) and maintenance requirements increased (sic)); thus the system was no longer optimal for maximum growth rate (Dixon et al. 1988).

Variation in the response to CO₂ with medium composition was observed in the case of Ps. fluorescens (Tan & Gill 1982). If basic cell functions, such as protein synthesis, or reactions of central metabolic pathways were the processes that became most rate-limiting for growth under CO₂, then the pattern of inhibition would be expected to be similar in the different media. As they were not it was necessary to postulate different rate-limiting reactions for each medium. It was postulated that the inhibition of growth rate by CO₂ would depend on either non-specific inhibition of enzymes involved in the early stages of substrate utilization or the inhibition of substrate uptake. Indirect inhibition of enzymes, resulting from a decrease in intracellular pH caused by CO₂ (Wolfe 1980), was untenable as an explanation since decreasing the intracellular pH by altering the medium pH actually stimulated growth (Tan & Gill 1982). The conclusion arrived at was that the inhibitory action of CO₂ was probably exerted at the level of substrate uptake. A point in favour of this conclusion was the failure of other workers to detect an accumulation of metabolites during CO₂-inhibited growth of Ps.
Neil M. Dixon and Douglas B. Kell

Fig. 1. Futile cycles, involving CO₂, that have been proposed as possible mechanisms of energy dissipation. (a) The futile cycle proposed for *Klebsiella aerogenes* (Teixeira de Mattos et al. 1984) which involves PEP carboxylase and PEP carboxykinase and is stimulated by CO₂. (b) CO₂ freely permeates the biological membrane and once inside the cell, CO₂ may then react with water to form bicarbonate ions and liberate protons. In an attempt to maintain the internal pH, protons are actively transported from the cell, resulting in the dissipation of energy. The bicarbonate ions may be actively transported from the cell, at a further energetic cost, or may ‘leak’ from the cell. As the hydration of CO₂ is reversible, the bicarbonate ions may react with a proton to produce water and CO₂, which is able to permeate the membrane thus completing the cycle. O₃ proton pump (ATP hydrolase); •, HCO₃⁻ pump or leak.

*Pseudomonas aeruginosa* (King & Nagel 1975), an organism closely related to *Ps. fluorescens*. Accumulation of some intermediate might be expected to result from the inhibition of specific catabolic enzymes, but this would not occur if the growth rate was being determined by the rate of substrate uptake. However, metabolites might not accumulate due to increased metabolic flux through other pathways that do not include CO₂ inhibited reactions. Additionally, substrate uptake may be expected to contribute to the growth rate-limitation but not be the sole reason for such limitation (see Dixon et al. 1987; Walter et al. 1987).

During the storage of proteinaceous foods in modified atmospheres containing CO₂, the interaction of CO₂ is achieved through solubilization and absorption of CO₂ (Mitsuda et al. 1975). Most probably, a major factor in the efficacy of CO₂ lies in its ability to penetrate the bacterial membrane, causing intracellular pH changes (Aickin & Thomas 1975; Turin & Warner 1977) of a greater magnitude than would be found for similar external acidification, which can be effectively buffered by the organism. The pH changes induced by medium-to-high partial pressures of CO₂ in the storage atmospheres are sufficient to disrupt the internal enzymatic equilibria (Wolfe 1980). Such internal pH changes may affect enzymes not otherwise involved with CO₂, and in addition to this, as all decarboxylation reactions appear to produce carbon dioxide in the CO₂(14) form (Krebs & Roughton...
1948; Delente et al. 1969) there is the possibility of feedback inhibition at elevated pCO₂. Reducing the temperature would serve to enhance this function by increasing the solubility of CO₂.

Mutants of E. coli, Neospora crassa and Salmo typhimurium that are specifically inhibited by CO₂ have been isolated (Roberts & Charles 1970). These mutants are inhibited at CO₂ concentrations which stimulate CO₂-requiring mutants and which do not inhibit the wild-type organisms, with the inhibition being relieved by the addition of specific growth substances; for example, the CO₂ inhibition of a methionine-requiring mutant of N. crassa is reversed by purines, and the CO₂ inhibition of a prototroph of E. coli is reversed by methionine or vitamin B₁₂.

Carbon dioxide may exert a direct influence upon enzymes by affecting their physico-chemical properties. The rate and extent of solution of proteins in water may be increased by CO₂, and in some cases insoluble proteins become soluble; this is reversible since they precipitate out again upon removal of the CO₂ (Mitz 1957, 1978). Hence CO₂ may be utilized to adjust the solubility of proteins during their separation and purification (Yanari et al. 1960). Changes in CO₂ tension may cause changes in the rate of solution, absolute solubility, dissociation of complexes, reactivity, stability, charge and configuration of proteins (Mitz 1978).

A property of CO₂, mentioned above, is that it has a high reactivity with amines (Edsall 1969). The reaction of CO₂ with uncharged primary amines is many times faster than with water, resulting in the formation of a carboxylic acid (RNH₂COOH), with different amines having different reaction rate constants. At equilibrium the amount of carboxylic is small, as the dominating reaction is the reaction with water, due to the relatively small concentrations of the proteins and the high concentration of water present.

As has been indicated CO₂ can react with amino acids, peptides and proteins of the cell. The protein carboxylate can cause the formation of internal electrostatic attractions or repulsions that could result in structural changes (Mitz 1979). These interactions could be between separate portions of the protein, between sub-units of a protein complex, or between the protein and bound heavy metals (Mitz 1979). Charge changes due to the reaction of CO₂ with amino groups, as mentioned above in the case of membrane proteins, could cause very important changes in the properties of the protein, especially in the case of enzymes which depend upon the possession of a specific charge for their activity. It is these structural and charge changes that may be responsible for the above-mentioned changes in the physico-chemical properties of proteins, caused by CO₂.

Alteration of the physico-chemical properties of a protein has been demonstrated in the case of haemoglobin (Kilmartin & Rossi-Bernardi 1973). CO₂ attached to the amino group of the protein as a carboxylate is associated with structural changes of the molecule which decrease the affinity for O₂. The ability of CO₂ to modulate the activity of enzymes has been demonstrated on enzymes in situ, in the case of enzymes of carbohydrate and fatty acid metabolism of rat liver (Hastings 1970; Longmore et al. 1974), as has the ability of CO₂ or bicarbonate to regulate enzyme activity in crude cell-solubles, e.g. NADP⁺-specific glycerol dehydrogenase (Legisa & Mattey 1986). Another example of this is the activation of RuBPCase by CO₂, mentioned above, resulting in the alteration of the enzyme’s sedimentation coefficient (Bowen & Gottschalk 1982).

12. Concluding remarks

Despite the large number of reports upon the inhibition by CO₂, which forms the basis of the discussion above, there does not appear to be a clear understanding of the role of CO₂ in the growth and metabolism of any given organism. Systematic studies of the effects of CO₂ on bacteria are therefore warranted. Such studies are of importance in attempting to understand: (1) the role of CO₂ in the preservation of foodstuffs, (2) the control by CO₂ of fermentations of biotechnological importance, and (3) the production of fine chemicals via reactions involving CO₂.

13. Acknowledgements

We are grateful to the Biotechnology Directorate of the Science and Engineering Research Council, UK, for financial support and to Jan Harris for proof-reading the manuscript. We thank John...
Hedger and Gareth Morris for stimulating discussions, and an anonymous referee for helpful comments.

References


BECKER, Z.E. 1933 A comparison between the action of carbonic acid and other acids upon the living cell. Protopenm 25, 161-175.

BELL, L. 1980 Gas in trunkers, containers helps packages keep foods fresh. Package Engineering 25, 72-75.


BREIDT, P.E. 1980 Use of controlled atmospheres to retard deterioration of produce. Food Technology 34, 45-50.


BROWN, W. 1922 On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and carbon dioxide. Annals of Botany 36, 257-283.


CASTELLI, A., LITTARU, G.P. & BARBI, G. 1969 Effect


HANSSON, G. 1982 Methane production from glucose and fatty acids at 55-85°C: adaptation of cultures.


Hunschke, J.H. 1988 Experimental approaches to determining carbon dioxide transfer in modified atmospheres. Food Technology September 55-64.


Hurst, A. 1981 Nisin. Advances in Applied Microbiology 27, 85-123.


Klei, H.E., Sundström, D.W. & Miller, J.E. 1984 Fermentation by Clostridium acetobutylicum under


Lioutas, T.S. 1988 Challenges of controlled and modified atmosphere packaging: a food company's perspective. *Food Technology* September 78–86.


Lorimer, G.H. & Mizorko, H.M. 1980 Carbamate formation on the e-amino group of a lysyl residue as the basis for the activation or ribulosebisphosphate carboxylase by CO2 and Mg2+. *Biochemistry* 19, 5321–5328.


OGILVY, W.S. & AYRES, J.C. 1953 Post-mortem changes in stored meats. V. Effect of carbon dioxide on microbial growth on stored frankfurters and characteristics of some microorganisms isolated from them. Food Research 18, 121–130.


SLATER, C. 1893 A bacteriological investigation of artificial mineral waters. Journal of Pathology and Bacteriology 1, 468-488.


SMITH, W.H. 1963 The use of carbon dioxide in the transport of fruits and vegetables. Advances in Food Research 12, 95-146.


CO₂ inhibition


THOMSON, J.E. & RISE, L.A. 1971 Dry ice in various shipping boxes for chilled poultry: Effect on micro-


Supplied by The British Library - "The world's knowledge"


