Effects of CO₂ on Budding and Fission Yeasts

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The budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* were grown under CO₂ pressure. Cell division was inhibited in both yeasts; RNA and protein content per cell declined but DNA continued to be synthesized and, in the case of *Sacch. cerevisiae*, reached twice the normal level within 24 h. Changes in mean cell volume occurred in both yeasts within 1 h; an increase occurred in the budding yeast while a decrease was observed in the fission yeast. The results suggest that CO₂ may exert its influence on cell characteristics through a change in cell volume.

INTRODUCTION

The effect of CO₂ on yeast metabolism and growth has not been widely studied. However, there is evidence which suggests that pressures of about 30 kPa upwards of excess CO₂ can exert marked effects on various characteristics of yeast. These effects have attracted interest from two distinct points of view. Firstly, by varying the head pressure (and hence the amount of dissolved CO₂) in fermenting vessels, the yield of various microbial products can be modified. For example, CO₂ has been used as a controlling agent in the brewing industry (Hoggan, 1980). Specifically, increasing the CO₂ pressure decreases the concentrations of many flavour compounds and esters during brewery fermentations (Drost, 1977) – the production of acetate esters, for example, is reduced (Norstedt *et al.*, 1975). Secondly, attention has been directed to the effects of CO₂ on the growth and division processes of yeasts. Jones & Greenfield (1982) reported that various pressures of CO₂ appeared to inhibit both the rate of yeast growth and the cell yield. An excess pressure of 197 kPa CO₂ markedly reduced cell yield (Arcay-Ledezma & Slaughter, 1984) while 282 kPa CO₂ inhibited cell division in *Saccharomyces cerevisiae* almost completely, although DNA replication continued, resulting in cells with twice the normal DNA content (Norton & Krauss, 1972). These workers also showed that the effects were not due to physical pressure as such, but to CO₂ in solution, as the effects did not occur when other gases were used instead of CO₂.

The present work is an attempt to evaluate the effects of CO₂ pressure on cell division and on basic cell characteristics of a budding yeast, *Sacch. cerevisiae*, and a fission yeast, *Schizosaccharomyces pombe*.

METHODS

Organisms. *Saccharomyces cerevisiae* NCYC 1108 and *Schizosaccharomyces pombe* NCYC 132 were maintained on slopes of malt extract agar.

Growth of cells. Yeast was inoculated from slopes into 10 ml sterile malt extract broth (Oxoid), at a concentration of 20 g l⁻¹. After 1 d, this starter culture was inoculated into 2 l sterile malt extract broth, and incubated with shaking for 3 d to obtain maximal cell yield. The cells were then harvested by centrifugation at 4 °C and 2500 r.p.m. for 15 min, and inoculated into 5 l sterile malt extract broth in a fermenting vessel at 25 °C.

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DNA and RNA estimation. Nucleic acids were extracted in perchloric acid, as described by Penman (1974), and assayed by the diphenylamine method (Burton, 1956). 2-Deoxyadenosine (Sigma) was used as the standard and the DNA value was calculated by multiplying by 2.605 (Bostock, 1966). RNA was assayed using orcinol (Trevelyan & Harrison, 1956), with *Torula* yeast RNA (Sigma) as the standard.

Protein estimation. The nitrogen content of the cells was estimated using the Tecator Kjeldahl nitrogen determination system, comprised of the Tecator Digestion System 6 and the Tecator Kjeltec System 1002 Distilling Unit. The nitrogen content was multiplied by 6.25 to obtain values for protein.

Cell size determination. Photographs were taken of the cell cultures ($\times 400$ magnification) at selected time intervals, and the cell length, diameter, area and volume were all measured using a magnetic tablet and cursor. The cursor and tablet were all measured using a magnetic tablet and cursor. The cursor and tablet were connected to a Kontron-MOP Videoplan computerized image analyser and printer. This equipment automatically calculated individual and average cell parameters and also calculated SD values.

Constant pressure fermenters. Fermentation vessels used were 11.5 l capacity stainless steel Cornelius 'Spartan' tanks (Cornelius, Anoka, Minnesota, USA). The CO₂ pressurized vessels were kept at a constant head pressure of 282 kPa above atmospheric pressure by means of a pressure release valve, whilst the controls were kept at atmospheric pressure (Arcay-Ledezma & Slaughter, 1984). Vessels were not stirred, but were swirled immediately before sampling to obtain a uniform cell suspension.

RESULTS

Cell numbers

Cultures (5 l) of both *Sacch. cerevisiae* and *Schiz. pombe* were grown for 24 h at 25 °C. The inoculum sizes were approximately 0.4×10^7 cells ml⁻¹ for *Sacch. cerevisiae* and 2.25×10^7 cells ml⁻¹ for *Schiz. pombe*. The inoculum sizes differed simply because the length of the pre-culture was the same for both types of yeast, namely 3 d, and after this time a higher yield of the fission yeast under the stated conditions was always achieved. Samples were removed every 2 h. Very little budding occurred in the *Sacch. cerevisiae* cultures grown under CO₂ pressure, and after 24 h there were about 0.5×10^7 cells ml⁻¹, while in the unpressurized control cultures there were approximately 1.7×10^7 cells ml⁻¹. A similar effect was observed in the *Schiz. pombe* cultures: after 24 h there were 2.15×10^7 cells ml⁻¹ in the pressurized cultures and 5.0×10^7 cells ml⁻¹ in the control cultures.

DNA and RNA contents

Fig. 1 shows the DNA contents of *Sacch. cerevisiae* and *Schiz. pombe*. After 24 h, the amount of DNA per cell of *Sacch. cerevisiae* had risen to approximately twice that calculated for the control cultures. The values of DNA per cell for the control (unpressurized) cultures remained fairly constant throughout the experiment, and were comparable with values obtained previously for brewers' yeasts, namely approximately 1×10^{-13} g per cell. The *Schiz. pombe* cultures also showed an increase in the value of DNA per cell in the CO₂ pressurized culture. The control value was approximately 3.5×10^{-14} g per cell, and rose to approximately 5×10^{-14} g per cell under CO₂ pressure.

The RNA content of the two types of yeast was slightly lower in cells grown under CO₂ pressure for 24 h.

Protein content

The Kjeltec nitrogen determination system allowed very reproducible results to be obtained for the two different types of yeast under both sets of conditions. In *Sacch. cerevisiae* cultures a transient increase in the amount of protein per cell was observed in cells grown under CO₂ pressure. This occurred during the first half of the culture period, but in the next 12 h the value fell to about 2.5×10^{-11} g protein per cell, as opposed to approximately $3.5\text{--}4.0 \times 10^{-11}$ g for the control cells. For *Schiz. pombe*, the amount of protein per cell was also lower after growth under CO₂ pressure but this was the case throughout the experimental period. The value after 24 h was about 1×10^{-11} g per cell under CO₂ pressure, and about 1.75×10^{-11} g for the control cells.

Fig. 1

Fig. 2

Fig. 1. DNA content of cells of *Sacch. cerevisiae* (a) and *Schiz. pombe* (b). □, ○, Cells grown at atmospheric pressure; ■, ●, cells grown under excess CO₂ pressure; ▲, cells grown under CO₂ pressure for 12 h followed by growth at atmospheric pressure.

Fig. 2. Average cell volume of *Sacch. cerevisiae* (a) and *Schiz. pombe* (b) for the first 2 h after inoculation. □, ○, Cells grown at atmospheric pressure; ■, ●, cells grown under excess CO₂ pressure.

Table 1. Effect of CO₂ on the number of budded cells in cultures of *Sacch. cerevisiae*

The yeast was grown in malt extract broth at 25 °C either unpressurized or under an excess pressure of 282 kPa CO₂.

Time (h)	Percentage of budded cells	
	Unpressurized	Pressurized
2	54	27
4	83	17
6	70	34
8	68	32
10	65	30
12	55	42
14	64	41
16	48	55
18	50	59
20	53	70
22	44	70
24	53	63

Cell size

The Kontron-MOP Videoplan system allowed rapid measurement of cell size data, automatically calculating the mean and SDM as each cell was measured. When *Sacch. cerevisiae* was subjected to CO₂ pressure over the 24 h sampling period, a greater mean cell volume was observed. The increase was complete within the first 2 h. After 2 h, the mean volume stabilized at approximately 250 μm³ compared to approximately 150 μm³ for control cells. The percentage of budding cells in the pressurized vessel was low for the first half of the culture period, but began to increase after 10–12 h (Table 1). This indicates that the CO₂ prevents cell budding in some manner, which may explain the increase in cell size if the cells continue to grow.

Similarly, in *Schiz. pombe* cultures fewer cells divided after being subjected to CO₂ pressure, but in this case the mean cell size was less. The mean volume of the *Schiz. pombe* cells subjected to CO₂ pressure decreased from approximately 50 µm³ to around 30 µm³. The decrease again occurred mainly within the first 2 h. The failure of the cells to reach a certain size may be a factor which stops them from dividing. When samples both of *Sacch. cerevisiae* and *Schiz. pombe* were taken every 15 min within the first 2 h period, cell size analysis revealed that the change in cell volume was very rapid. Change was detectable within the first 15–30 min for both types of yeast (Fig. 2), and in both cases the mean cell volumes remained constant after 2 h.

Recovery after pressurization

It was of interest to check if the observed effects of CO₂ on the cells were permanent, or if they could be reversed, i.e. if the cells could revert to normal growth and metabolism. Cells were cultured under pressure for 12 h, the pressure released, and the cells cultured for a further 12 h at atmospheric pressure. Samples were then checked for cell numbers, DNA and RNA contents, protein content and cell size. The cells began to recover from the CO₂-induced changes, although normal nucleic acid and protein contents and cell size were not achieved by 24 h. A possible reason for this could be that although the applied pressure was released the medium would still contain appreciable amounts of dissolved CO₂.

DISCUSSION

In our experiments cell division in *Sacch. cerevisiae* was greatly inhibited by an excess pressure of CO₂ and the DNA content per cell was approximately doubled. Cell division was also inhibited in the fission yeast *Schiz. pombe*, and DNA content per cell increased, although within the same experimental period the increase was less than in *Sacch. cerevisiae*. The reductions in RNA and protein content found under CO₂ pressure are consistent with a general drop in metabolic activity. The experiments in which the pressure was released after 12 h clearly show, however, that these changes cause no irreversible damage. Morphologically, the failure of *Sacch. cerevisiae* cells to divide appears to be due to an inhibition of bud formation, as indicated by the relatively small numbers of budded cells in culture. This view is supported by the observed overall increase in mean cell volume; such an increase would occur if cell wall material was still being synthesized. Since chitin is a major component at the constriction point between mother cell and bud in budding yeasts (Marchant & Smith, 1968; Cabib & Bowers, 1975), it is possible that some type of interference in the synthesis of the chitin ring would prevent the cells from budding. However, fission yeasts like *Schiz. pombe* divide by elongative growth followed by cell separation by invagination or 'waisting' in the centre of the elongated organism. One of the key events in this type of division process is the formation of the septum, the major constituent of which is 1,3-β-glucan (Duffus *et al.*, 1984). If interference in the synthesis of chitin rings or septa is the major factor inhibiting cell division, the effects of CO₂ pressure seem biochemically different in the two organisms. Alternatively, CO₂ may be inhibiting an earlier event in cell division which is common to both yeasts.

The first observed effect of CO₂ pressure in the two yeasts, the changes in cell volume, were complete within the first 2 h of culture. It is therefore possible that the other effects, namely changes in nucleic acid and protein contents, are secondary to the initial changes in cell size. Cell size controls over DNA synthesis and nuclear division have been proposed to regulate cell division in fission yeasts (Fantes & Nurse, 1977). The mechanism involved in the changes in cell size and the consequences for cell metabolism of this change are clearly of great potential significance in the study of control of cell function and especially cell division.

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