Effects of Compression with Ethane, Ethylene and Their Fluorinated Derivatives on Yeast Growth

S Kawachi, T Arao, Y Hara, Y Suzuki and K Tamura
Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
E-mail: tamura@chem.tokushima-u.ac.jp

Abstract. The inhibitory effect of compressed gaseous C2 compounds on yeast growth was investigated quantitatively by microcalorimetry. The growth thermograms (heat output vs. incubation time) showed that all C2 compounds tested inhibited yeast growth. After quantification of yeast growth at various pressures, we determined the 50% inhibitory pressure (IP50) and the minimum inhibitory pressure (MIP) as indices which represent the inhibitory potency of gases. The lower the IP50 and MIP values, the greater the growth inhibitory effects of the gases. Based on these values, the inhibitory potency of the gases increased in the order: ethane (C2H6) < ethylene (C2H4) < pentafluoroethane (C2HF5) < 1,1,1,2-tetrafluoroethane (C2H2F4). Furthermore, transmission electron microscopy (TEM) of yeast cells treated with compressed ethylene showed that the inner structures of the cells, especially the nuclear membrane and cytoplasmic membrane, were damaged.

1. Introduction
Gaseous C2 hydrocarbons and their fluorinated derivatives are widely used in industry. Ethane is one of the components of natural gases and ethylene is used as a starting material for the production of plastics and other basic chemicals and produced in the largest volume among organic materials [1]. Some of C2 fluorinated hydrocarbons are also available as alternative fluorocarbons. For example, HFC-134a that is a representative of C2 fluorinated hydrocarbons, has found many applications ranging from refrigerants to blowing agents [2]. In future, these C2 compounds will be more increasingly used. Although there is a risk of human exposure to C2 gaseous compounds, little information is available on toxic properties of these gases.

Recently, we have proposed a new method to quantify the toxicity of various gases through observing the inhibition of yeast growth under high-pressure gases [3]. Generally, at normal pressure, the solubilities of gases such as oxygen, nitrogen, hydrocarbon gases are low in aqueous systems. It is therefore difficult to evaluate the effects of gases on yeast growth in aqueous medium. In order to solve this problem, we pressurized gases to increase their concentrations in yeast cultures and evaluated the inhibitory effects of gases on yeast growth by microcalorimetry. Using this method, we investigated the toxic effects of ethane, ethylene and their fluorinated derivatives on yeast growth. Additionally, the effects of compression with ethylene on ultrastructure of the yeast were examined by transmission electron microscopy (TEM).
2. Materials and Methods

The yeast strain employed in the present study is *Saccharomyces cerevisiae* IFO10149. The log-phase cultures of yeast were pressurized with the experimental gases and then incubated in fresh YPD medium at 30°C. A multiplex isothermal batch calorimetry (Biothermo Analyzer, Nippon Medical and Chemical Instruments, type H-201) was used to monitor yeast growth under high-pressure gases.

For transmission electron microscopy (TEM), the cells were prefixed with 2% glutaraldehyde, postfixed with 1.2% potassium permanganate, dehydrated with alcohol series and embedded. Ultrathin sections were observed with transmission electron microscopy (Hitachi, H-7000).

3. Results

3.1. The growth thermograms under compressed gaseous C2 compounds

We show the growth thermograms obtained from growth cultures under four kinds of C2 gases at various pressures (figure 1). In these experiments, normal pressure is indicated as 0 MPa. All the calorimetric signals increased due to the growth of yeast, reached a peak, and finally returned to baseline due to nutrient depletion and accumulation of metabolites. It is obvious that the initial slope of the growth thermograms decreased and that the peak shifted towards a longer incubation time with increasing pressure for each gas. These results indicate that all gases employed showed an antimicrobial effect over the range of pressures utilized in this study.
3.2. IP\textsubscript{50} and MIP values for gaseous C2 compounds

The inhibitory effects of all gases used are clear in decreasing the growth rate of yeast and increasing the incubation time required for the cultures to reach a certain activity level. The inhibitory effects of yeast growth at various pressures, called the specific growth activity, were quantified from the growth rate constant and growth duration time. The specific growth activities at various pressures were fitted after they were plotted against gas pressure. See other literatures for more details on the quantification of growth inhibition [3]. We determined both the 50\% inhibitory pressure (IP\textsubscript{50}), which reduces the growth of yeast by 50\% and the minimum inhibitory pressure (MIP) at which yeast growth is completely inhibited. The 50\% inhibitory pressure, IP\textsubscript{50}, corresponds to the gas pressure that results in specific growth activity of 0.5 in the fitted curve. The minimum inhibitory pressure, MIP, is defined as the gas pressure that yields a specific growth activity of zero in the fitted curve. The lower the IP\textsubscript{50} and MIP values, the greater the growth inhibitory effects of the gases. Based on these values, the order of potency for growth inhibition was ethane < ethylene < pentafluoroethane < 1,1,1,2-tetrafluoroethane (table 1).

| C2 compounds            | Formula  | IP\textsubscript{50} / MPa | MIP / MPa | Growth rate constant | Growth duration time |
|-------------------------|----------|----------------------------|-----------|----------------------|----------------------|
| Ethane                  | C\textsubscript{2}H\textsubscript{4} | 0.776 | 1.75 | 0.856 | 1.80 |
| Ethylene                | C\textsubscript{2}H\textsubscript{4} | 0.607 | 0.832 | 0.681 | 0.925 |
| Pentafluoroethane       | C\textsubscript{2}HF\textsubscript{5} | 0.396 | 0.703 | 0.296 | 0.640 |
| 1,1,1,2-Tetrafluoroethane | C\textsubscript{2}H\textsubscript{2}F\textsubscript{4} | 0.130 | 0.253 | 0.110 | 0.242 |

3.3. Effects of compressed ethylene on the ultrastructure of S. cerevisiae

We also observed changes in intracellular organelles after exposure to compressed ethylene, using TEM. Treatment with compressed ethylene resulted in severe damage to the inner structures of the cells as shown in figure 2b. Nuclear membrane pores opened (figure 2b, single arrow) and vacuolar membrane was disrupted. The cell membrane invaginated deeply into the cytoplasm (figure 2b, double arrows) and an electron-light area in the cytoplasm was observed.

![Figure 2](image_url)

**Figure 2.** Transmission electron microscopy (TEM) images of *Saccharomyces cerevisiae* grown in YPD medium without (a) and with treatment with ethylene at 0.640 MPa (b). CM, cell membrane; CW, cell wall; N, nucleus; V, vacuole; bars, 1 \( \mu \)m.
4. Discussion

In this study, the inhibitory effects under gaseous C2 compounds on yeast growth were investigated quantitatively by microcalorimetry. The MIP values of ethane, which showed the least inhibitory effect on the yeast growth, were less than 2 MPa. In contrast, complete inhibition of yeast growth was achieved with hydrostatic pressure of approximately 50 MPa [4]. These results indicate that growth inhibition under gaseous C2 compounds is caused by dissolved gases. Moreover, we found that the inhibitory potency of the gases increased in the following order: ethane < ethylene < pentafluoroethane < 1,1,1,2-tetrafluoroethane. The introduction of double bond or fluorine atoms into a paraffin compound increased the inhibitory potency of C2 compounds whereas the number of fluorine atoms did not depend on their inhibitory potency. There are some reports that unsaturated bonds or halogen atoms increase their solubilities in lipid membrane and their anaesthetic potency [5-7], and it has been found that the toxicity of hydrocarbons or halogenated alkanes depends on their hydrophobicity [8,9]. Similarly, the inhibitory effects of our gaseous C2 compounds would be associated with their solubilities in lipid or their hydrophobicity. In addition, it has been reported that a maximum hydrophobicity and anaesthetic potency were reached when the fluorination and hydrogenation were well balanced [6]. Therefore, 1,1,1,2-tetrafluoroethane could exhibit more inhibitory effect on yeast growth than pentafluoroethane.

In our morphological studies using TEM analysis, we observed changes in intracellular organelles after treatment with compressed ethylene. These changes appear to be mainly due to the inhibitory potency of ethylene. Decompression of ethylene is another possibility of damage, however, the decompression has less influence on yeast because of low pressure of 0.640 MPa. The type of cell damage when exposed to compressed ethylene is different from the appearance of holes in cell wall or cell burst observed after gas decompression [10]. Ethylene has been reported to inhibit the glucose transport system of yeast, which is one of the functions of membrane [11]. Thus, compressed ethylene is considered to have considerable effects on the structural and functional properties of membranes. Moreover, the structural effects of compressed ethylene on yeast cells occurred on the nucleus, suggesting that it caused the cells incapable of growing. Although these results were limited to those after treatment with ethylene, similar results could be obtained when treated with other C2 compounds. It has been described that fluoroalkanes also might interact with membranes, causing a change of membrane permeability [12]. More work is necessary to clear the toxic mechanisms of C2 compounds.

5. References

[1] Nath R and Alzein 2000 Comput. Chem. Eng. 24 533-38
[2] Tsai W T 2005 Chemosphere. 61 1539-47
[3] Arao T, Hara Y, Suzuki Y and Tamura K 2005 Biosci. Biotechnol. Biochem. 69 (7) 1355-71
[4] Iwahashi H 2000 Nippon Nogeikagaku kaishi. 74 609-11
[5] Hunter A R 1962 Brit. J. Anaesth. 34 224-8
[6] Eger E I, Liu J, Koblin D D, Laster M J, Taheri S, Halsey M J, Ionescu P, Chortkoff B S and Hudlicky T 1994 Anesth. Analg. 79 245-51
[7] Eger E I and Laster M J 2001 Anesth. Analg. 92 1477-82
[8] Sikkema J, Bont de J A, Poolman B 1995 Microbiol. Rev. 50 (2) 201-22
[9] Akers K S, Sink D G and Schultz T W 1999 Environ. Toxicol. Pharmacol. 7 33-39
[10] Nakamura K, Enomoto A, Fukushima H, Nagai K and Hakoda M 1994 Biosci. Biotechnol. Biochem. 58 (7) 1297-1301
[11] Thomas K C and Spencer M 1978 Can. J. Microbiol 24 222-27
[12] Auken O W and Healy J 1975 Can. J. Microbiol. 21 221-226

Acknowledgments

We thank Dr. M Osumi and Dr. Y Hataba (Integrated Imaging Research Support) for TEM analysis. This study was supported in part by a Grant-in-Aid for Scientific Research (C) (20510025) from the Japan Society for the Promotion of Science.