MICROORGANIZMS INACTIVATION IN THE MEDIUM OF SUPERCRITICAL OR LIQUID CO_2.

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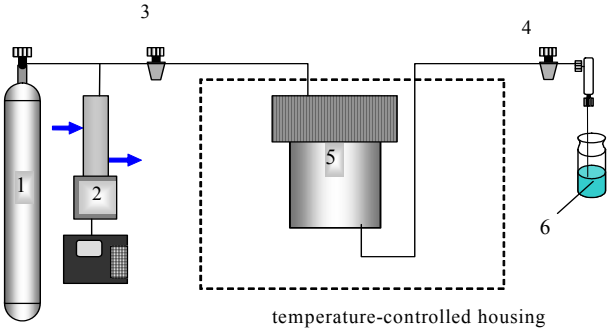
The relationship between the properties of bacteria extremophiles and the effectiveness of their inactivation in the carbon dioxide medium was demonstrated.

INTRODUCTION

Obviously, sterilization should provide complete destruction of pathogenic bacteria. Three main procedures are often used for treatment of medical facilities, namely, treatment with ethylene oxide, γ-ray irradiation, and steam sterilization. All three procedures are highly effective in destruction of pathogenic bacteria. At the same time they are characterized by certain disadvantages, which are related with the fact that the sterilized materials should retain their main mechanical and physicochemical properties. As proposed in [1], treatment in supercritical (AC) and compressed (C) CO_2 at temperatures which would not affect the properties of the sterilized materials seems to be a promise method providing high effectiveness of the bacteria destruction. In this work we studied the effect of carbon dioxide medium on the growth of bacteria extremophiles.

MATERIALS AND METHODS

Bacteria Culturing**.** The bacteria *Escherichia coli* AB1157 were cultivated in a LB-medium incubated for 18–20 h at 37°C. The bacteria *Deinococcus radiodurance* R1 (most resistant among know virus strain) was cultivated at 37°C in a twice diluted LB-medium. All cells were grown at the Division of Molecular and Radiation Biophysics (St. Petersburg Institute of Nuclear Physics) directly before the experiments to ensure operation with fresh grown-up cells.
The prepared cell cultures were dispensed in the small laboratory microvials (200 μl) and precipitated on a centrifuge (10000 rpm) for 5 min. The supernatant liquid was removed and the vials with precipitated cells arranged in the containers were placed in a high pressure unit of the experimental installation. The installation for sterilization and sterilization high pressure unit are shown in Figs. 1 and 2, respectively. In all the experiments the sample were treated in the CO_2 medium for 20 min at 34°C, whereas the working pressure was varied from 20 to 140 atm.



1- cylinder with compressed CO_2
2- high-pressure pump with control unit
3, 4 - valves
5 – high-pressure unit
6- collector of extract

→ - water cooling of the pump cylinder (T=12°C)

Fig. 1. Installation for sterilization in CO_2 atmosphere

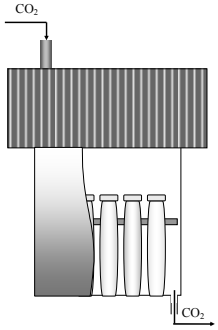


Fig. 2. High-pressure sterilization unit

The stainless steel high-pressure unit was designed so to provide prolonged cycles related with pressure increase and revealing without any removal of the cell cultures and pollution of the high-pressure pipes owing to reverse diffusion.

In each experiment, four laboratory small laboratory vials containing fresh cell cultures were place in the high pressure unit. The unit was sealed and heated in a temperature-controlled housing to 34°C. Then the unit was filled with CO_2 up to working pressure, carbon monoxide was preliminarily heated to 34^0C using a special heater. After sterilization the pressure was revealed in 5-7 min, and the samples were removed from the high-pressure unit.

The sterilization effect of the SC and C CO_2 was evaluated by the ratio of the amount of living cells (N) after treatment to the amount of cells initially added to the unit (No). The number of living cells was determined before and after the experiment by counting the amount of growing cells (cfus) on the selective agar plates. The triplicate results were averaged for each data point.

RESULTS and DISCUSSION

In was found SC CO_2, in combination with appropriate installation and corresponding experimental procedures provide an effective method of sterilization for thermally and hydrolytically labile biomedical materials by inactivation of a variety of bacterial microbes.

In our work, the following bacteria were used in the supercritical fluid sterilization procedure: *E. coli* AB1157 and *Deinococcus radiodurance* R1. These bacteria are widely spread in the nature and known data on their properties suggest that they are extremophiles resistant to radiation. In all experiments the high-pressure unit was charged with at least 10^7–10^8 cfu/ml of the microorganism. In all the experiments we determined the limiting parameter (pressure or time) was determined at which no cfus were formed after incubation for 48 h. Complete sterilization of all the microorganisms studied occurred in 20 min at 80 bar and at temperature 34°C (see Figure 3). It should be noted that the experiments were performed in the absence of organic solvents, reagents, or γ-ray irradiation. Though inactivation of some bacteria cultures can require another pressures and times, enhanced mass transfer would significantly reduce these parameters. The inactivation kinetics can be significantly improved by changes in the unit design to increase the time and effectiveness of interaction between the bacterial material and fluid. The inactivation kinetics for wet cells *E. coli* and *D. radiodurance* are given in Figure 3.There are no significant differences in the sensitivity of these bacteria to the treatment with compressed CO_2. This fact suggests that the time of complete inactivation of wet cells *E. coli* and *D. radiodurance* at their treatment in CO_2 medium at pressure ≥ 7 MPa is smaller than 20 min. The time of complete in=activation in SC CO_2 is significantly smaller than that at sterilization with ethylene oxide or γ-ray irradiation (from several hours to day) and at steam treatment (at 120^0C and higher).

It is assumed that inactivation of the microorganisms in supercritical CO_2 in the presence of small amounts of water (predominantly, intercellular water) proceeds owing to penetration of carbon dioxide through the cell wall [3] and subsequent interaction with intracellular element. However, along with high penetrability of SC CO_2 we should take into account changes in the

medium pH (to 2.8) of the equilibrium water phase owing to formation of carbonic acid [4]. Hence, carbonization of intracellular water and interaction of carbonic with the cell elements probably causes the cell deactivation [5-8].

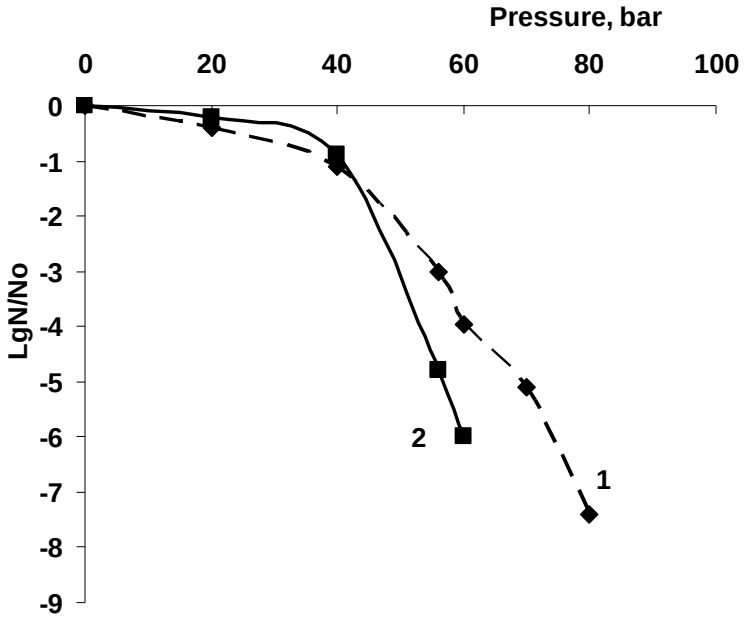


Fig. 3: Inactivation of wet cells as a function of CO_2 pressure
(1) *D. Radiodurance* and (2) *E. coli.*

CONCLUSION

Our experimental data suggest that the wide use of sterilization in supercritical or compressed CO_2 is hindered is hindered by the absence of data on the process parameters providing complete inactivation of all the polluting microflora. This suggests that the study of inactivation of most widely spread microorganisms including those exhibiting extremal properties (extremophiles) in supercritical and compressed carbon dioxide in comparison with common sterilization procedures such as autoclave treatment, γ-ray irradiation, and treatment with ethylene oxide is of particular importance.

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