Effects of moderate pressure on premeability and viability of *Saccharomyces cerevisiae* cells

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Abstract−With CO_2 and N_2 as the pressure media, the effects of the moderate pressure (0.1-1.0 MPa) and the holding time on the conductivities of the cell suspension of *Saccharomyces cerevisiae* CICC1447 and *Saccharomyces cerevisiae* CICC1339, as well as the absorbances of the supernatant (after centrifuged) at 280 nm (A_{280}) and 260 nm (A_{260}) were determined. The membrane permeability of *Saccharomyces cerevisiae* CICC1447 increased significantly and the cell leakage was aggravated with the pressure increase. For *Saccharomyces cerevisiae* CICC1339, the conductivity of the cell suspension, A_{280} and A_{260} of the supernatant fluctuated with the pressure increase; as a whole, they increased with pressure. Different from high pressure, a moderate pressure not only remarkably improved the permeability of the yeast cell membrane, but also kept yeast cell viability; moreover, the integrity of the yeast cell membrane could be maintained.

Key words: Moderate Pressure, *Saccharomyces cerevisiae*, Permeability, Viability

INTRODUCTION

Despite the early beginnings of high pressure processing technology, in commercial terms there have been relatively few applications of high pressure for microbial inactivation in foods and accumulating intracellular metabolites of general physiological stress (such as trehalose, glutathione). Although high-pressure processing technology is available on a commercial scale, it still is relatively high cost. Thus, it is necessary to develop a new method for microbial inactivation in foods and accumulating intracellular metabolites of general physiological stress. The influence of high pressure on living organisms associated with food and intracellular metabolites has been investigated [1,2]. To understand the impact of high pressure on cell viability, different approaches have also been developed. The results from some investigations showed that high pressure (more than 10 MPa) brought about significant changes in a microorganism's morphology [3,4], metabolic flux [5,6], gene expression [7,8] and the permeability of the cell membrane etc. [9,10,12,13]. The changes of the high pressure may also alter the osmotic pressure between the cell membrane [8,9]. However, there are few reports about the effects of the moderate pressure (0.1-1.0 MPa) on the bioactivity of microorganism cells [14]. Thus, it is important to investigate the effect of the moderate pressure on the bioactivity of microorganism cells. Yeasts are considered a good model for stress response investigations. Among them the budding yeast *S. cerevisiae*, which is an easy to grow and genetically well accessible microorganism, belongs to the family of ascomycetes. As also many other foodspoilage fungi belong to this class of fungi, we considered this yeast a suitable model organism for the study of the mode-of action and physiological effects of moderate pressure. Thus, the main aim of this work was to investigate the effects of moderate pressure (0.1-1.0 MPa) on the cell permeability of *S. cerevisiae* with N_2 and CO_2 as the pressure media. It was found from this study that the moderate pressure (0.1-1.0 MPa) not only remarkably improved the permeability of the yeast cell membrane, but also maintained yeast cell viability; moreover, the integrity of the yeast cell membrane could be maintained. These results can help people to develop some new methods for microbial inactivation in foods and accumulating intracellular metabolites of general physiological stress (such as trehalose, glutathione).

MATERIALS AND METHODS

1. Pressurizable Bioreactor

The pressurizable bioreactor system used is shown in Fig. 1. The

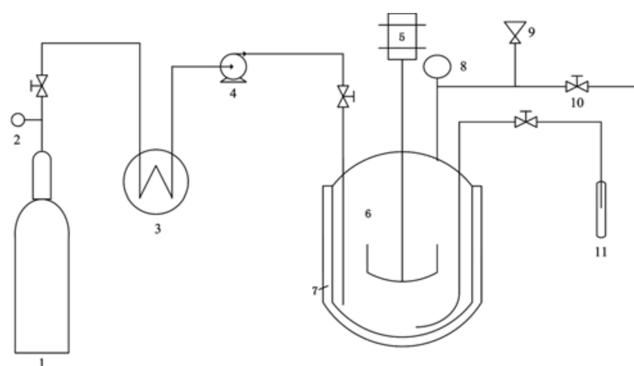

Fig. 1. Schematic of pressurizable bioreactor system configuration.

1. Gas cylinder
2. Pressure gauge
3. Cold trap
4. High pressure pump
5. Stirrer
6. Reactor
7. Water jacket
8. Pressure gauge
9. Safety valve
10. Snuffle valve
11. Sample tube

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reactor was made of stainless steel which consisted of a magnetic stirrer, a cylindrical vessel with a volume of 300 ml (internal diameter: 60 mm) and a water jacket for the temperature control. For all compression experiments presented here, the CO_2-inlet or N_2-inlet was at the base of the vessel in order to sparge the gas through the suspension. The pressure was supplied with a high-pressure pump and the compression rate was 0.05 Mpa/min and the decompression rate 0.05 Mpa/min.

2. Strains and Media

Two *S. cerevisiae* strains, *S. cerevisiae* CICC1447 and *S. cerevisiae* CICC1339 preserved in our laboratory were used as test strains. *S. cerevisiae* CICC1339 was purchased from china general microbiological culture collection center (CGMCC2.2080), *S. cerevisiae* CICC1339 was treated with ultraviolet to obtain *S. cerevisiae* CICC 1447.

Seed cultivation medium (YEPD) contained the following (g/L): glucose, 20; yeast extract, 10; peptone, 20; pH, 6.0. The composition of fermentation medium (g/L) is as follows: glucose, 40; yeast extract, 10; peptone, 20; $MgSO_4$, 1.0; KH_2PO_4, 1.0; pH, 6.0.

3. Culture of *S. cerevisiae* Cells

Two loops of *S. cerevisiae* were inoculated into the seed medium and grown in a rotary shaker (HZSH-H water bath rotary shaker, made by Haerbin Donglian Electronic Technology LTD. China) at 120 r/min for 24 h at 30 ºC as inoculum culture. 10 ml of inoculum culture was added into a 500 ml Erlenmeyer flask containing 90 ml of the fermentation medium. The temperature was controlled at 30 ºC in a rotary shaker at 120 r/min for 24 h of scale-up culture [15,16].

4. Preparation of the Cell Suspension

The fermentation broth of the scale-up culture of *S. cerevisiae* was centrifuged at 4,000 r/min for 10 min; then the cells were washed twice with double distilled water, and then the harvested cells were weighed and suspended in the double distilled water (2%, g/g) as cells suspension [11].

5. Pressure Treatment

50 ml cell suspension diluted with double distilled water (approx. 10^7 cells/ml) was put in the preheated 0.3 *l* pressurizable bioreactor (designed by our laboratory) at 30 ºC, and then the reactor was pressurized with N_2 or CO_2 (99.99%, Tianjin Boke gas industry Ltd., China). The control sample was treated with the same procedure above except that it was kept at atmospheric pressure. When the reactor was decompressed, the samples were immediately taken out for analysis.

6. Analytic Methods

The conductivities of control samples and pressurized samples were measured with a DDA-11A conductivity meter (Shanghai Analytical Instrument Company, China). After the conductivity was measured, the cell suspension was centrifuged at 4,000 r/min for 10 min; then the protein and nucleic acid concentrations in the supernatant were measured according to the absorbance at 280 nm (A_{280}) and 260 nm (A_{260}) with ultraviolet light (UV-2401PC Shimadzu Co. Ltd., Tokyo, Japan) respectively [10]. The morphology of the *S. cerevisiae* cells was observed with scanning electron microscope (SEM, PHILIPS XL-30, Holland). The viability of *S. cerevisiae* cells was determined by the colony-forming units (CFU) method.

The Coulter® Epics® XI™ Flow Cytometer manufactured by Beckman Coulter Company (USA) was employed to conduct the fluorescence activated cell sorter analysis (FACS).

RESULTS AND DISCUSSION

1. Effect of Moderate Pressure on A_{280} and A_{260} and Conductivity of *S. cerevisiae* Cell Suspension

When suspended in the double distilled water, *S. cerevisiae* cells were in a hypotonic situation. The ions and other inclusions in the cells such as nucleic acids and proteins can leak out continually to make the conductivity, A_{280} and A_{260} of the cell suspension increase

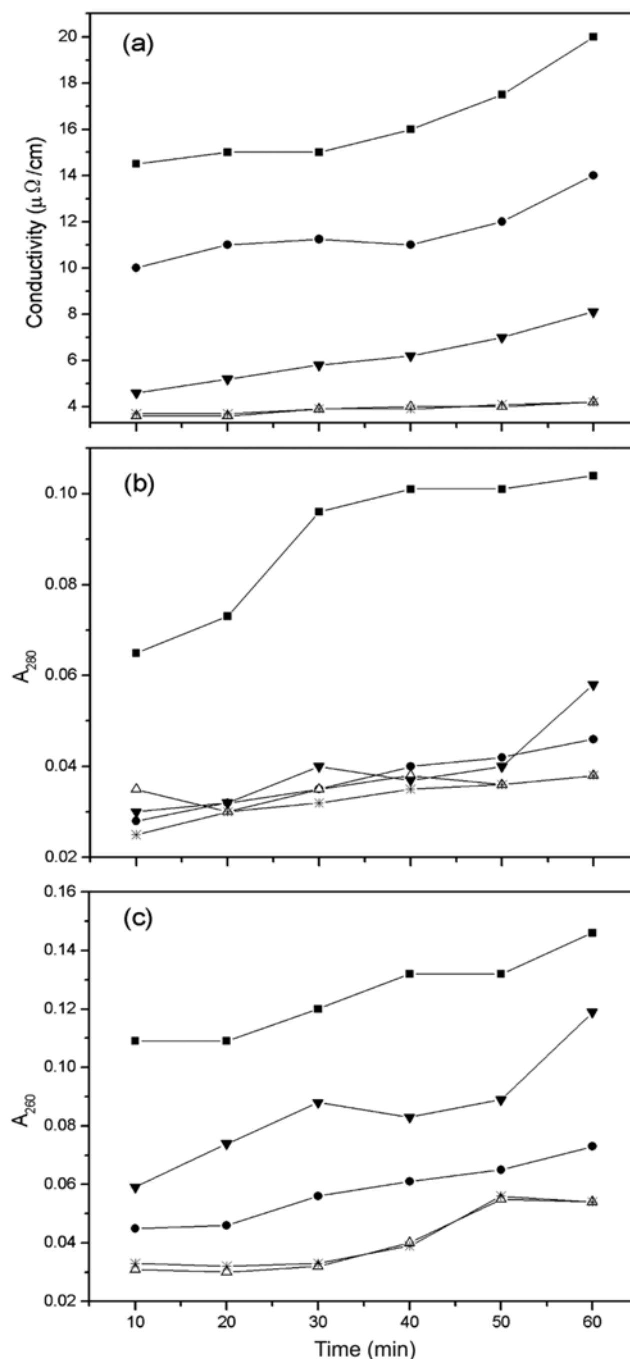

Fig. 2. Changes of the membrane permeability of CICC1447 cells with time under different pressure medium. (a) Conductivity; (b) A_{280}; (c) A_{260} (■, 1.0 MPa of CO_2; ●, Ordinary pressure of CO_2; △, Ordinary pressure of air; ▼, 1.0 MPa of N_2; *, Ordinary pressure of N_2).

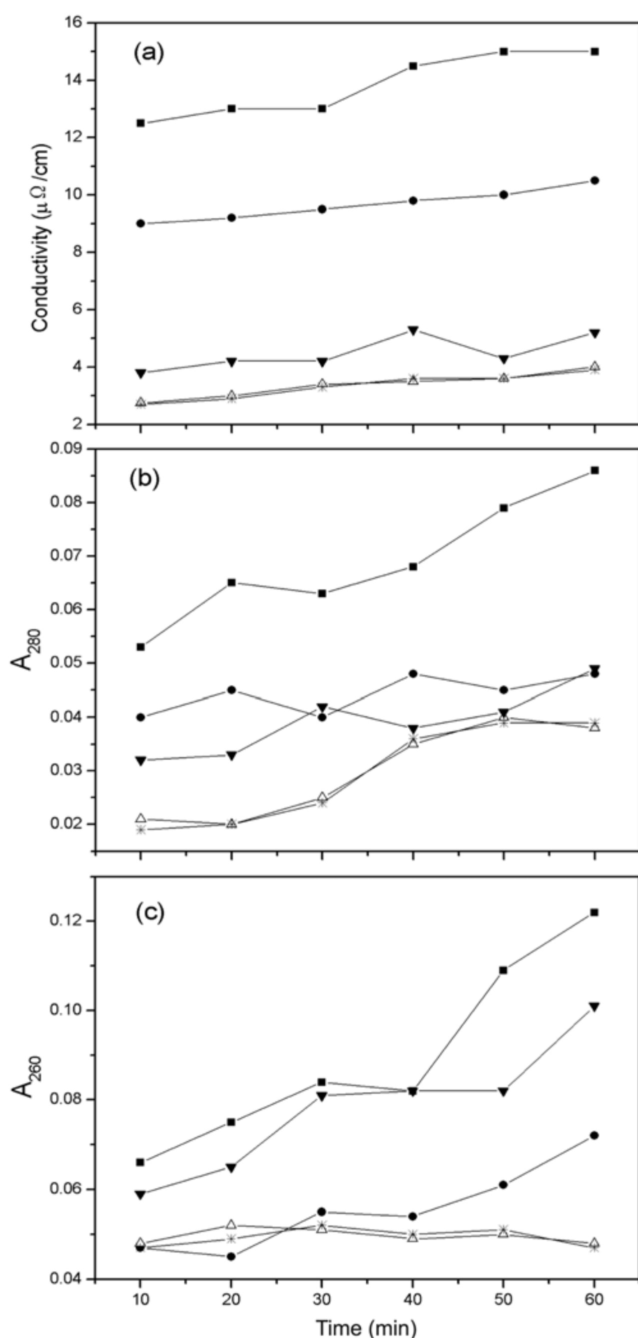

Fig. 3. Changes of the permeability of CICC1339 cells with time under different pressure medium. (a) Conductivity; (b) A_{280}; (c) A_{260} (■, 1.0 MPa of CO_2; ●, Ordinary pressure of CO_2; △, Ordinary pressure of air; ▼, 1.0 MPa of N_2; *, Ordinary pressure of N_2).

with time [10], and this tendency could be intensified by the pressure (Figs. 2 and 3). With 1.0 MPa of CO_2 as the pressure medium, obvious improvement of the conductivity, A_{280} and A_{260} of the cell suspension was observed for CICC1447 and CICC1339. Similar results were also obtained with 1.0 MPa of N_2 as pressure medium and t-test showed that there were significant differences for CICC1447 in the conductivity (P=0.0074), A_{280} (P=0.000025) and A_{260} (P=0.0045) between the experimental group of 1.0 MPa of N_2 experiments and the control group of ordinary pressure. It was also analogous for that of CICC1339. The pressure with N_2 as the pressure medium improved the membrane permeability of CICC1339 cells, and significant differences were observed in the conductivity (P=0.000015), A_{280} (P=0.0036) and A_{260} (P=0.0036) between the experimental group under 1.0 MPa of N_2 of pressurized experiments and the control group. These results indicated that the 1.0 MPa of moderate pressure with N_2 as the medium could alter the permeability of *S. cerevisiae* cells.

When CO_2 is employed as a pressure medium, partly solved CO_2 combines with H_2O to form H_2CO_3. H_2CO_3 disassociates as expressed by Eqs. (1) and (2)

$$H_2CO_3 = H^+ + HCO_3^- \quad (1)$$

$$HCO_3^- = H^+ + CO_3^{2-} \quad (2)$$

Because H^+, HCO_3^- and CO_3^{2-} could penetrate through the cell membrane and promote the effect of pressure on cell permeability, these ions themselves also have conducting ability in water; the cell activity may be lowered when CO_2 is used as pressure medium. Therefore, N_2 was employed as the pressure medium and the pressure was maintained for 30 min to study the effect of pressure on the permeability of *S. cerevisiae* cells. The changes of the conductivity, A_{280} and A_{260} of the cell suspension are shown in Figs. 4 and 5. It revealed that the cell permeability of CICC1447 increased significantly with the pressure and the cell leakage aggravated, but for CICC1339, the suspended liquid conductivity, A_{280} and A_{260} fluctuated with the pressure; as a whole, they increased with pressure.

The samples of *S. cerevisiae* CICC1339 and *S. cerevisiae* CICC 1447 cultured to the stationary phase and treated under 1.0 MPa of moderate pressure for 1 h were taken as the experimental samples. The samples treated under ordinary pressure were taken as the control samples. All the samples were colored with propidium iodide (PI), and the fluorescence intensities were analyzed by Flow cytometry. The results are shown in Tables 1, 2.

As an ordinary fluorescence dye, PI gives fluorescence from the combination of DNA. In the coloring process, PI penetrates through

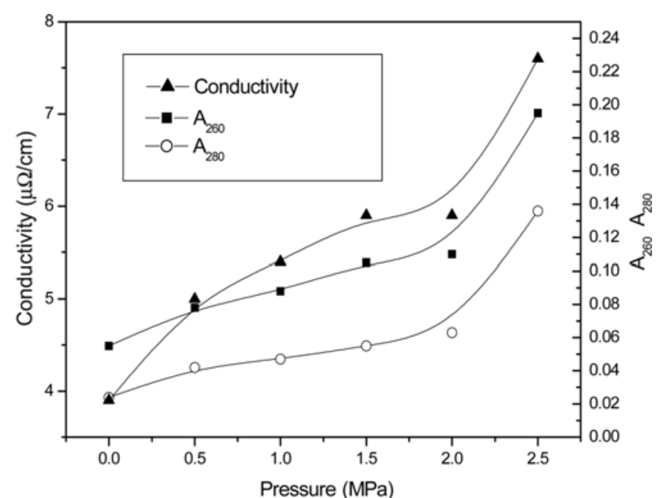

Fig. 4. Effect of moderate pressure on the permeability of CICC1447 cells (N_2 as the pressure medium).

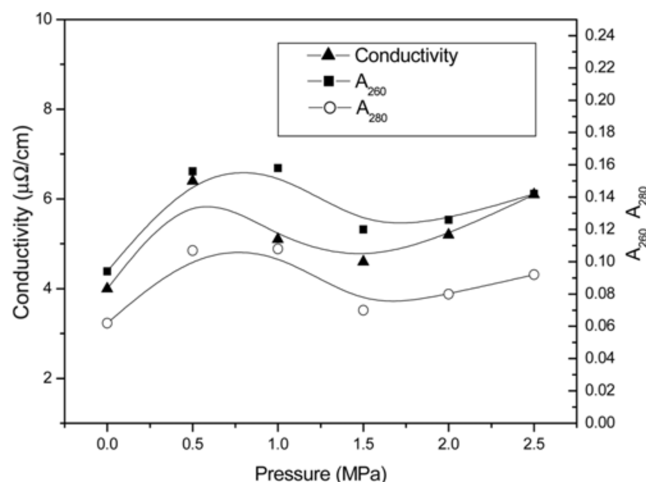

Fig. 5. Effect of moderate pressure on the permeability of CICC1339 cells (N_2 as the pressure medium).

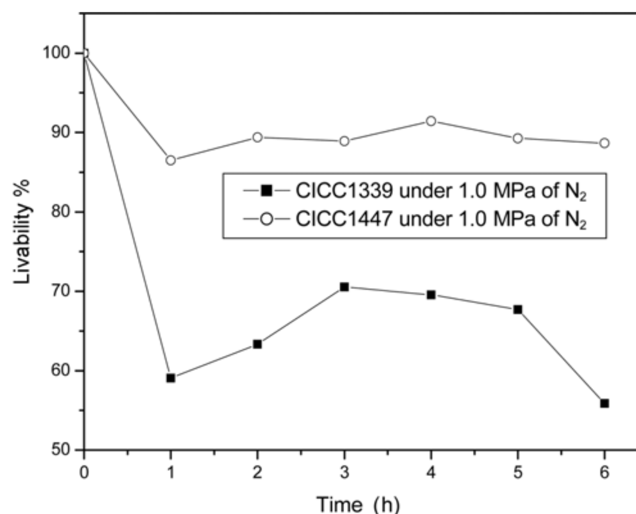

Fig. 6. Effect of moderate pressure on viability of *S. cerevisiae* cells.

Table 1. Comparison of fluorescence intensities of CICC1339 cells under different pressure and time

Pressure	Fluorescence intensity	
	1 h	3 h
Ordinary pressure	0.649	0.732
1.0 MPa of N_2	0.669	0.782

Table 2. Comparison of fluorescence intensities of CICC1447 cells under different pressure and time

Pressure	Fluorescence intensity	
	1 h	3 h
Ordinary pressure	2.32	2.58
1.0MPa of N_2	2.47	2.93

the cell membrane into the cell and combines with DNA molecules. If the cell membrane is damaged and the permeability increased, PI can enter the cell more easily and the fluorescence intensified. Thus, the changes of the fluorescence intensity of the colored cells can be used to determine the permeability changes of cells. If the fluorescence intensity of *S. cerevisiae* cells increases under pressure, it indicates that the pressure can degrade the integrity or increase the permeability of *S. cerevisiae* cells. Table 1 and 2 show that the average fluorescence intensities of the experimental group were higher than that of the control group for both CICC1339 and CICC1447. For example, the mean fluorescence intensities of CICC1447 cells (Table 2) under ordinary pressure for 1 h and 3 h were 2.32 and 2.58, respectively; however, they increased to 2.47 and 2.93 under 1.0 MPa of N_2 for 1 h and 3 h correspondingly. That is, the mean fluorescence intensities of the experimental group under pressure increased 6% and 13.56%, respectively. These results indicate the 1.0 MPa of moderate pressure remarkably improved the permeability of the *S. cerevisiae* cells. It accords with the result from the measurement of A_{280} and A_{260} and conductivity.

2. Effect of Moderate Pressure on Viability of CICC1447 and CICC1339

The effect of moderate pressure on the viability of CICC1447 and CICC1339 cells under 1.0MPa of N_2 was investigated by the colony-forming units (CFU) method (Fig. 6). The results showed that CICC1447 cells had higher viability than that of CICC1339 cells under 1.0 MPa of N_2. It is well-known that trehalose is a membrane protector and acts like other membrane protectors that induce tolerance to pressure. Several papers have shown the importance of trehalose content for cell protection against the deleterious effect of pressure [17-20]. In previous reports, we found that trehalose content of *S. cerevisiae* CICC1447 was higher than that of *S. cerevisiae* CICC1339 when *S. cerevisiae* CICC1447 and *S. cerevisiae* CICC1339 were treated in moderate pressure [21]. The more likely explanation was that *S. cerevisiae* CICC1447 cells synthesized better trehalose and therefore protected cell against the deleterious effect of pressure, which increased the high viability of CICC1447 cells. Brul et al. [10] thought that the breakage of the cell membrane integrity under the high pressure (300 MPa) is responsible for the inactivation of the *S. cerevisiae* cells. However, it can be seen from our experiments under moderate pressure that CICC1339 cells, which have smaller changes in permeability, are more sensitive to pressure in viability. On the contrary, CICC1447 cells, which have larger changes in permeability, have smaller change in viability. Therefore, it may be considered that *S. cerevisiae* 1447 cells with higher permeability are more tolerant to pressure. As a whole, it is different from that under high pressure; the viability of *S. cerevisiae* cells can be maintained, while the permeability of the cell membrane increases under moderate pressure.

3. Effect of the Moderate Pressure on the Morphology of *S. cerevisiae* Cells

From the SEM observation, we can see that the moderate pressure made rugae form on the surfaces of *S. cerevisiae* cells. The cells of CICC1447 deformed obviously; however, the cells of CICC 1339 kept the original appearance. Combined with the result in Fig. 7, it can be deduced that the rugae formed under pressure protected the cells from damaging; thus, *S. cerevisiae* CICC1447 has higher resistance to pressure than CICC1339. In general, they maintained integrity under moderate pressure.

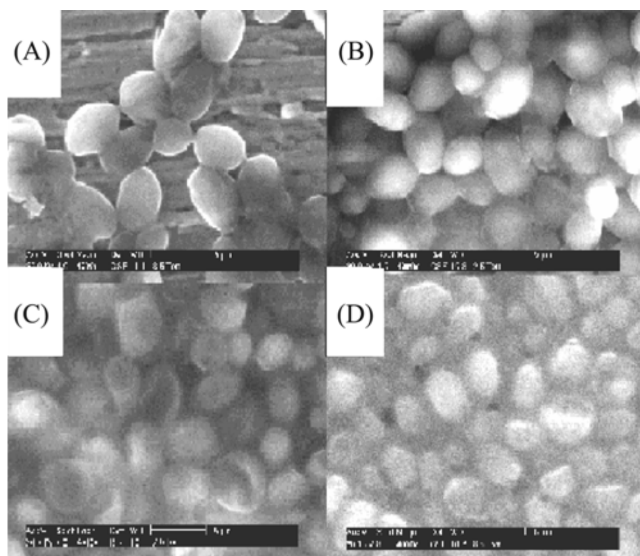

Fig. 7. Sem images of CICC1339 and CICC1447 cells. (A) CICC 1447 under 1.0 MPa, (B) CICC1447 under 0.1 MPa, (C) CICC1339 under 1.0 MPa, (D) CICC1339 under 0.1 MPa.

CONCLUSION

In moderate pressure, the membrane permeability of *S. cerevisiae* CICC1447 increased significantly and the cell leakage aggravated with the pressure increase. CICC1339 cells, which have smaller changes in permeability, are more sensible to pressure in viability. Namely, the moderate pressure not only improved the permeability of the yeast cell membrane, but also kept yeast cell viability; moreover, the integrity of the yeast cell membrane can be maintained. It is indicated that this novel approach (moderate pressure) for microbial inactivation in foods and accumulating intracellular metabolites of general physiological stress has much applied value in the biotechnology.

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