

STRESS AND STRAIN IN A YEAST CELL UNDER HIGH HYDROSTATIC PRESSURE

Christoph Hartmann*, Antonio Delgado*

*Chair of Fluid Dynamics and Process Control, Technische Universität München, Freising, Germany

Summary The mechanical effects of the compression of a yeast cell (*Saccharomyces cerevisiae*) under high hydrostatic pressure (HHP) are modeled and simulated numerically. Dimensional analysis shows that oscillatory pressure application at frequencies below 700 MHz does not generate mechanical damage. The deformation of the cell under pressure deviates strongly from isotropic volume reduction. In the cell wall, von-Mises stress reaches the critical value upon failure at a pressure load between 415 MPa and 460 MPa.

INTRODUCTION

The treatment of food and biotechnological substances with hydrostatic pressures up to 1000 MPa (i. e. 10000 bars) has manifold effects such as phase transitions, the unfolding of proteins, the inactivation of enzymes, the preservation of aroma compounds and vitamins. High pressure induced inactivation of micro-organisms is investigated intensively on a molecular level, but little is known about the mechanical effects on living cells and the resulting morphological changes [1]. Morphological changes of yeast cells (*Saccharomyces cerevisiae*) under high hydrostatic pressure (HHP) with damage of cell organelles prior to cell wall disruption at pressure levels between 400 MPa and 500 MPa are reported in [2]. The analysis is carried out ex-situ, while in-situ observation of cells exposed to high hydrostatic pressure is still a challenge due to the strong pressure dependency of optical properties. The difficulty of combining a microscopic device with a thick-walled high pressure chamber and the severe requirements on the chamber material are additional handicaps. Moreover, an in-situ observation of cell organelles seems even to be impossible at present. Therefore, the present contribution intends to deliver a mathematical model and a simulation of the mechanical effects during the high hydrostatic pressure treatment of *Saccharomyces cerevisiae* (baker's yeast). This organism is chosen, because it is widely used in food and biotechnology. Furthermore, the organism is of a certain mechanical complexity since it has a cell wall, a membrane and organelles. The paper principally focuses on the distribution of stress and strain within the cell, their correlation to experimental findings and the question, whether transient pressure application protocols can lead to mechanical damage of the cell.

METHODS

Geometry and Structural Elements

In order to describe the mechanical behavior of the cell, the complex structure of the cell is reduced to an axis-symmetric model containing the relevant structural parts of the cell: the cell wall, the cytoplasm (contained in the cell), the nucleus and the vacuole. Other cell organelles are supposed to be of minor importance for the mechanical behavior of the cell. Membranes around the whole cell, the nucleus and the vacuole are taken into account as material surfaces. The geometrical shape of the yeast cell model represents a cross section through an ellipsoid with an imperfection at the bottom representing the bud scar. It contains a spherical nucleus and a spherical vacuole placed along the axis of symmetry at distinct locations. The typical length of the cell is 10 μ m (Figure 1).

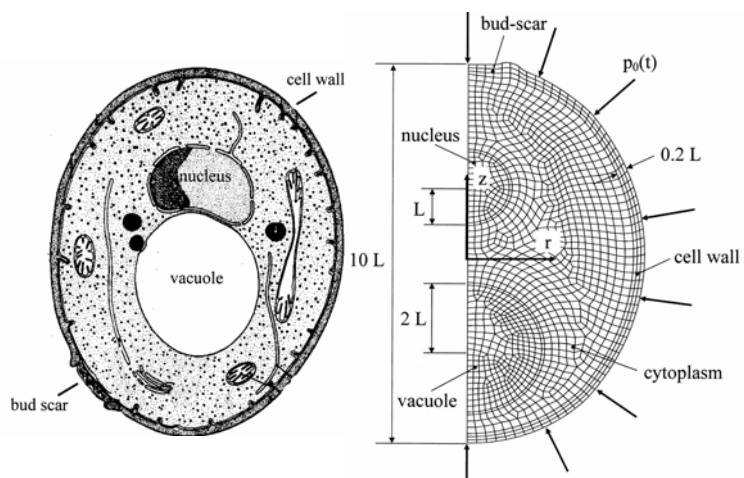


Figure 1: Structure and model of the yeast cell

Governing Equations and Solution Technique

The Cauchy equation of motion for a continuous medium forms the basis of the mathematical model of the present problem. Time dependent pressure load is applied as boundary condition on the outer surface of the cell wall. It is shown by dimensional analysis that the time dependent problem reduces to a stationary one. Since mechanical properties of the individual organelles of the yeast cell are difficult to determine, the current model accounts for simplified material properties of the cytoplasm (a gel filling the voids inside the cell), a fat-containing vacuole and the cell wall. The mechanical properties of the cell wall are taken from [3], where Young's modulus of the cell wall material is determined as $E_{cw} = 150 \text{ MPa} \pm 15 \text{ MPa}$ assuming almost incompressible, linear elastic behavior. Analysis of current model parameters as well as physical properties of cytoplasm suggests that the mechanical properties of cytoplasm exposed to high pressure can be approximated with those of pure water. Therefore, bulk modulus of water is

derived from an equation of state valid under high pressure and is shown to depend linearly on pressure. Analogously, bulk modulus for the lipid contained in the vacuole is determined from published thermodynamic data. The governing equations are solved with finite element methods. We use 1322 axis-symmetric 8-node-solid-elements in the whole computational domain. As shown by dimensional analysis, the problem can be considered to be a static load case. The pressure load is applied incrementally in order to achieve converged solutions at 10 load steps. Successive refinement of the computational mesh did not significantly influence the results.

RESULTS

Our simulation results are compared to experimentally determined volume reduction at pressures up to 250 MPa published in [4]. Generally, it can be stated that the data exhibit a good overall agreement, where the maximum deviation does not exceed 6%. This is most probably related to the fact that experimental volume measurements are based on the assumption that the cells have a spherical shape.

Application of a pressure load of 400 MPa generates a strong deformation of the initially spherical vacuole and nucleus (Figure 2). The overall shape of the cell approaches a spherical shape. The inner part of the cell remains in a hydrostatic stress state with zero von-Mises stresses. The maximum von-Mises-stress is located in the cell wall around $z = 0$ (coordinated system see fig. 1) and adopts values of up to 64 MPa. The authors of [2] observe ex-situ that the membranes of the organelles are all disrupted after a high pressure treatment at 400 MPa at ambient temperature. The outer shape of the organisms remains unaffected in this case. The present contribution reveals that at any pressure level maximum effective strain values are located in the membranes of the organelles. According to the observations in [3] an estimate of the critical effective strain of $\varepsilon_{VM,f} = 0.8$ upon failure of the organelle membranes can be delivered from the present load case at 400 MPa.

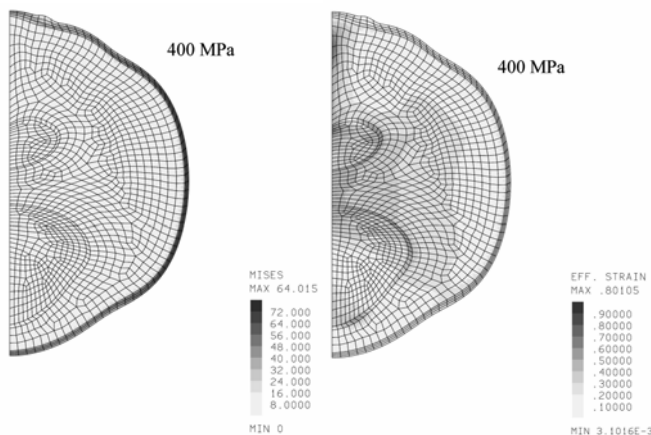


Figure 2: von-Mises stress and effective strain distribution of the 400 MPa load case

Ex-situ investigations of yeast cells treated at pressures of 400 MPa and 500 MPa [2], and critical values of the von-Mises stress upon failure for the cell wall material [3] at ambient non-hydrostatic conditions can be correlated to the results of the present analysis. The authors of [2] observe disruption of the cell wall at pressures above 400 MPa, while a von-Mises stress for failure of the cell wall is given in a range of $\sigma_{VM,f} = 70 \pm 4$ MPa [3]. Figure 2 shows a maximum von-Mises stress of 64 MPa in a 400 MPa load case being slightly below this critical range. Latter is completely covered at a pressure load between 415 and 440 MPa.

In addition to that, it can be concluded from the dimensional analysis of the mechanical problem, that transient pressure application cycles in a high hydrostatic pressure process do not generate any dynamic response of the cell. Structural inactivation of the cells (e. g. damage of the cell wall or membranes) is a result of static load on the cell and can not be enhanced by transient pressure application. The latter has to be expected if the coefficient of the inertia term of the non-dimensional equation of motion takes on values of the order of magnitude of one corresponding to a frequency of approximately 700 MHz. This value is far beyond the range of current application in HHP processing.

CONCLUSION

Referring to the questions formulated in the introduction following conclusions can be drawn: The interior of the cell is exposed to hydrostatic stress conditions as long as the material properties of the organelles do not differ much from those of the cytoplasm. The cell organelle membranes are strongly deformed prior to any damage of the cell wall. Pressure loads between 415 MPa and 460 MPa induce mechanical cell wall damage. Compared to constant pressure application, transient pressure protocols in HHP processing do not enhance the mechanical action on the yeast cells. A dynamic response of the cells can only be expected at pressure oscillation frequencies superior to 700 MHz.

References

- [1] Hartmann, C., Delgado A.: Numerical Simulation of the Mechanics of a Yeast Cell under High Hydrostatic Pressure. *J. of Biomechanics*, in press, 2004
- [2] Shimada, S., Andou, M., Naito, N., Yamada, N., Osumi, M., Hayashi, R.: Effects of hydrostatic pressure on the ultrastructure and leakage of internal substances in the yeast *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology* **40**:123-131, 1993.
- [3] Smith, A.E., Moxham, K.E., Middelberg, A.P.J.: Wall material properties of yeast cells. Part II. Analysis. *Chemical Engineering Science* **55**:2043-2053, 2000.

- [4] Perrier-Cornet, J. M., Maréchal, P. A., Gervais, P.: A new design intended to relate high pressure treatment to yeast cell mass transfer. *J. of Biotechnology* **41**:49-58, 1995.