

#### Available online at www.sciencedirect.com



Bioelectrochemistry

Bioelectrochemistry 65 (2004) 59-68

www.elsevier.com/locate/bioelechem

# Strong static magnetic field effects on yeast proliferation and distribution

Masakazu Iwasaka<sup>a,\*</sup>, Masateru Ikehata<sup>b</sup>, Junji Miyakoshi<sup>c</sup>, Shoogo Ueno<sup>a</sup>

<sup>a</sup>Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-Ku, Tokyo 113-0033, Japan

<sup>b</sup>Environmental Biotechnology Laboratory, Environmental Engineering Division, Railway Technical Research, 185-8540, Japan

<sup>c</sup>Department of Radiological Technology, School of Health Science, Faculty of Medicine, Hirosaki University, 66-1 Hon-cho, Hirosaki 036-8564, Japan

Received 24 June 2003; received in revised form 1 April 2004; accepted 8 April 2004 Available online 25 August 2004

#### **Abstract**

The present study focuses on the effects of gradient magnetic fields on the behavior of yeast, such as its proliferation and mass distribution, and evaluates the effects of magnetism on materials in the yeast culture system. Yeast, *Saccharomyces cerevisiae*, was incubated in a liquid medium under magnetic fields (flux density B = 14 T). When yeast in a tube was exposed to 9-14 T magnetic fields with a maximum flux density gradient of dB/dx = 94 T/m, where x is the space coordinate, the rate of yeast proliferation under the magnetic fields decreased after 16 h of incubation compared to that of the control group. The physical properties of the yeast culture system were investigated to discover the mechanism responsible for the observed deceleration in yeast proliferation under magnetic fields. Gas pressure inside the yeast culture flask was compared with and without exposure to a magnetic field. The results suggested that the gas pressure inside a flask with 6 T, 60 T/m slowly increased in comparison to the pressure inside a control tube. Due to the diamagnetism of water (medium solution) and yeast, the liquid surface distinctly inclined under gradient magnetic fields, and the hydrostatic force in suspension was strengthened by the diamagnetic forces. In addition, magnetophoresis of the yeast cells in the medium solution exhibited localization of the yeast sedimentation pattern. The roles of magnetically changed gas-transport processes, hydrostatic pressures acting on the yeast, and changes in the distribution of the yeast sedimentation, as well as the possible effects of magnetic fields on yeast respiratory systems in the observed disturbance of the proliferation are discussed.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Magnetic field; Yeast proliferation; Yeast distribution; Magnetic force; Diamagnetism; Moses effect

## 1. Introduction

Recent advances in superconducting technology have provided new methods of studying the application of strong magnetic fields in biology and soft material sciences as well as in solid state physics. Diamagnetism is the induced magnetism of all materials, and strong magnetic fields at room temperature reveal drastic behaviors of diamagnetic materials such as water and protein [1–4]. Room temperature bores of superconducting magnets have given both physicochemists and biologists opportunities to recognize and explore the importance of the diamagnetism of biological materials [4–6] as well as that of strong (ferro-) magnetism in solid state materials. Gradient mag-

netic fields in the horizontal direction generated several interesting phenomena. The parting of water, or the so called "Moses Effect," a phenomenon in which the surface of water under magnetic fields of the Tesla order separated, was observed in our laboratory [2]. The horizontal gradient magnetic force and the vertical gravitational force generate a sloping gravity-like field for living creatures [3], in which the sloping field is believed to be the actual direction of gravity. In the case of the parting of water, the water was pushed to lower magnetic fields from higher fields, and localized in the middle part of the space forming a "water-wall".

The present study focuses on the correlation of waterrelated magnetic field effects and biological processes. The biological functions and the genetic information of yeast are well understood, and it is possible to determine the origin or mechanism of magnetic field effects on yeast.

In the present study, we investigate the effects of gradient magnetic fields on yeast behavior, proliferation,

<sup>\*</sup> Corresponding author. Tel./fax: +81-3-5841-3601. *E-mail addresses:* iwsaka-m@umin.ac.jp, iwasaka@medes.m.u-tokyo.ac.jp (M. Iwasaka).

and distribution of yeast sedimentation. Yeast growth depends on the physical properties of the surroundings, such as temperature, dissolved oxygen concentration, and pressure [7–9] in water, as well as biological parameters. It is known that strong and high-gradient magnetic fields have an effect on gas absorption and desorption rates in an aqueous solution. However, the effects of dissolved gas concentrations and/or pressures of liquid—gas systems on cellular growth under magnetic fields have not been investigated in past studies. Our hypothesis is that the yeast in a liquid—gas mixture system under gradient magnetic fields should undergo mechanical effects via the magnetism of materials.

#### 2. Materials and methods

## 2.1. Yeast preparation and observation of proliferation

Yeast, Saccharomyces cerevisiae, was pre-cultured on a YPD agar plate, then moved to a peptone-glucose medium for a one-night incubation in a water bath with shaking. The yeast suspension was then diluted with peptone-glucose medium (0.4 wt.% Peptone (BACTO PEPTONE, DIFCO Laboratories, Detroit MI 48232-7058, USA) and 2 wt.% D-Glucose (Wako, Osaka, Japan)), then was homogeneously divided into eight to ten sample solutions in sterile 15-ml cylindrical centrifuge tubes (Corning, 430766). Each tube contained 3 ml of the yeast suspension. The sterile 15-ml cylindrical tubes containing the yeast suspension in logarithmic phase were exposed to magnetic flux densities (B = 5– 14 T) with a maximum gradient of 94 T/m (Fig. 1). In order to expose the tube to a wide range of gradient magnetic fields, the tube was set at an angle of 15° with reference to a horizontal line to compare yeast proliferation with magnetic field exposure to that without magnetic field exposure at 30 °C. The magnetic field exposure system, superconducting magnet, has a leakage of magnetic fields. The control experiments were conducted at a location about 4 m from the magnet, where B = 0.15 mT.

The density of the yeast suspension was measured after dispersing the yeast homogeneously throughout the suspension. Light absorption of the suspension at a wavelength of 600 nm was measured after incubations with and without the magnetic field exposures. After the yeast was dispersed in the suspension, the number of cells in suspension was counted to confirm the dependence of light absorption on cell density.

## 2.2. Observation of yeast sedimentation

Yeast sedimentation profiles were observed using an optical microscopic observation system, which consisted of a bore scope (Olympus, R080-124-090-50), a lens holder unit (Kogaku, Osaka, Japan) and a CCD color camera (Tokyo Electric Industry, CS5720). The bore scope had a

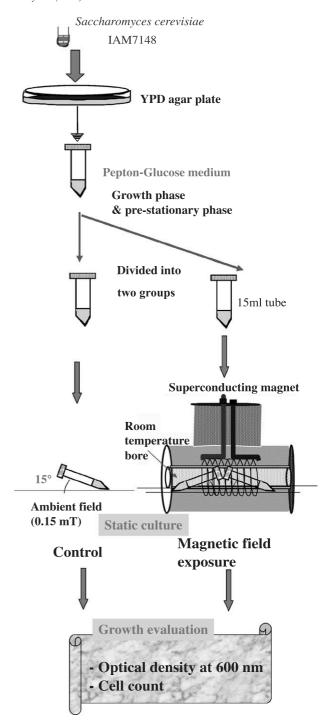


Fig. 1. Experimental procedures for observing the proliferation of yeast under magnetic fields. Upper: preparation of yeast before the magnetic field exposure experiments. Center: configuration of a superconducting magnet with a maximum magnetic flux density of 14 T and placement of the yeast culture tube in the magnetic fields. Bottom: using two methods to evaluate the proliferation of yeast: optical density at wavelength 600 nm and cell count.

mirror at the edge and introduced a side-view of the sterile 15-ml cylindrical tube, which contained 3 ml of yeast suspension, to the CCD camera via the lens. We injected a homogeneously suspended yeast suspension into the tube,

and used the microscope system to observe the process of yeast sedimentation under gradient magnetic fields.

## 2.3. Gas pressure measurements

A differential manometer was used to measure the gas pressure of the atmosphere above the yeast suspension. Fig. 2 is a diagram of the manometer with tubing. One tube was introduced into the yeast suspension inside the magnet (measurement) and the other was placed into the yeast suspension outside the magnet (reference). The manometer, Okano Works, Digital Micro Manometer DP-10A, detected a difference in pressure between the measurement tube and the reference tube. The tubes were each 3 m long and 10 mm in diameter and made of vinyl chloride. The terminus of the tubing was connected to the inlet of the yeast culture flask. During yeast proliferation, CO<sub>2</sub> gases were generated and the gas pressure inside the flask continuously increased. When both yeast culture flasks were incubated under the same conditions without exposure to magnetic fields, the gas pressures in the flask were balanced. It was expected that if the yeast proliferation under a magnetic field did or did not exceed that of the control, that the indicated values in the differential

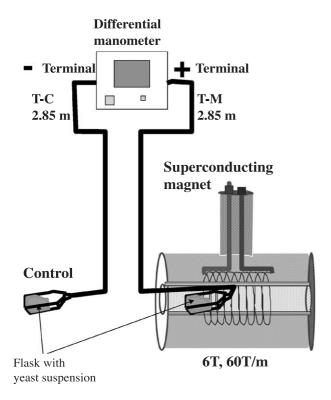


Fig. 2. Experimental setup for measurement of gas pressure inside yeast culture flask (NUNC 1 52094). The positive terminal of the differential manometer was connected by tubing to the inlet of the flask exposed to the magnetic field and the negative terminal was connected to the inlet of the control flask. The tubing for the control (T-C) and that for the magnet (T-M) were each 2.85 m long.

manometer would increase or decrease, respectively. The yeast culture flask was set in a magnetic field (B=6 T, dB/dx=60 T/m).

## 2.4. Centrifugal experiments

Two milliliters of yeast suspension was inserted into a 2-ml centrifugal tube, and the tubes were set at the rotator or at the bottom of the compartment of the centrifuge. The temperature of the compartment was 22 °C, and the centrifugation was carried out at 1000 rpm. After 16 h of incubation with and without centrifugation, the optical absorbance of the yeast suspension at wavelength 600 nm was measured.

## 2.5. Modulation of oxygen supply by tube settings

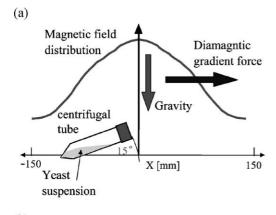
A 15-ml centrifugal tube with 9 ml of yeast suspension was set with its long axis parallel or perpendicular to the earth's gravity, and the effects of changing the altitude of the liquid were examined. In addition, the effect of covering the tube with a cap on yeast proliferation was observed to determine the effects of oxygen from the atmosphere. We compared the optical density (wavelength 600 nm) of the yeast suspensions in a capped tube (closed system) and in a tube covered with a film (open system) after incubations with and without magnetic field exposures. The film used was PARAFILM (American National Can TM, Chicago, IL 60631), which was utilized to prevent water dripping down the tube and which had more gas permeability than the cap. The tubes were set at angles of 60° and 15° from horizontal.

#### 3. Results

## 3.1. Optical density of yeast suspension

Fig. 3 shows an example of the effects of magnetic fields at 14 T on the yeast suspension. The experiments were conducted using yeast suspensions with an initial optical density at wavelength 600 nm (OD=0.1 and OD=0.4). The density of the yeast suspension increased during 14 to 19 h due to yeast proliferation. After 14 h, the yeast suspension (initial OD=0.4) showed a decrease under 14 T while no distinct difference was observed in the suspension (initial OD=0.1).

The effects of the magnetic field on yeast suspension that were observed were reproducible, as shown in Fig. 4, whose bottom portion shows the difference of optical density between the suspension in the presence and absence of the 14-T magnetic fields. The maximum effect of the magnetic field was observed 16 h after the beginning of both magnetic field exposure and incubation at 30 °C. The optical density of the suspension became saturated after 21 h and the effects of the magnetic field on the optical density diminished in comparison to the density at 16 h.



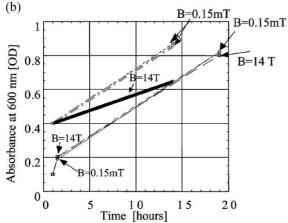
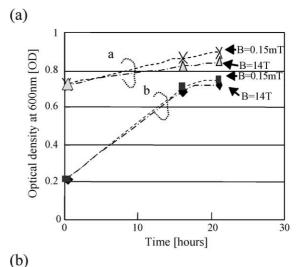


Fig. 3. Effects of magnetic fields of up to 14 T on the optical density of yeast suspension at wavelength 600 nm. The optical densities of the pair of sample tubes were measured after static incubation with and without exposure to magnetic fields. The upper panel shows the configuration of magnetic force acting on the diamagnetic solution and yeast, the direction of gravity, and the yeast culture tube. The bottom panel shows an example of the effects on the optical density of the yeast suspension.

Fig. 5 summarizes a series of measurements of the optical density of yeast suspension at wavelength 600 nm. After the individual incubation of a pair of samples with and without exposure to a magnetic field, the yeast suspensions were homogenized and their optical densities (ODs) were measured by a spectrophotometer. The difference in optical density ( $\Delta$ OD600) was obtained by subtracting the OD of the suspension under the magnetic field from that not subjected to the magnetic field. The results indicated that the optical density of the yeast suspension decreased under the magnetic fields, and also suggested that there was a deceleration in yeast proliferation. After 20 h of incubation, there was no difference because of the growth saturation of the yeast under the restricted condition of O<sub>2</sub> supplement. The differences in OD of the yeast with and without the influence of magnetic fields suggest that a delay in yeast proliferation occurred under the magnetic field.

In another experiment, to confirm the effect of the magnetic fields on yeast proliferation, the numbers of yeast cells with and without magnetic fields were counted on glass



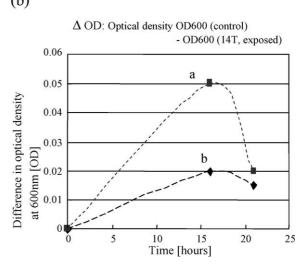


Fig. 4. Reproducible effects of magnetic fields of up to 14 T on the optical density of the yeast suspension at wavelength 600 nm. The bottom panel shows the difference in optical density (OD) between the exposed sample and the control. The optical density of group-a and group-b at the start was 0.73 and 0.2, respectively.

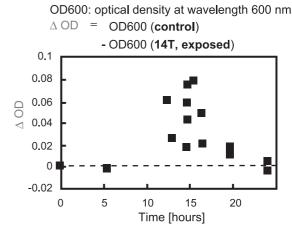


Fig. 5. Time dependence of the difference in optical density (OD) between the sample exposed to a 14-T magnetic field and the control. During 24 h, 12 sets of yeast culture tubes were taken from the incubator and their optical densities measured.

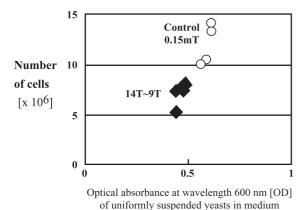


Fig. 6. Correlation of optical density of the yeast suspension with and without exposure to a magnetic field to the number of cells, which was counted on an optical microscope after 16 h of exposure to a magnetic field at 9 to 14 T.

slides under an optical microscope (Fig. 6). After 16 h of incubation, the proliferation rate of yeast under a magnetic field was distinctly less than that of the control group.

#### 3.2. Yeast sedimentation

Sedimentation of yeast particles occurred after 1 h of static placement. Fig. 7 shows the sedimentation profile of yeast in a peptone—glucose medium when the tube was set parallel to the horizontal line and exposed to gradient magnetic fields of 5 to 6 T. As the culture tube was filled to 25% with the medium, the medium containing the yeast was pushed down toward lower magnetic fields. The medium solution that primarily consisted of water showed a "water-wall", a declining boundary between the medium and both air and the yeast sedimentation. The changes of the density distribution of the yeast in the gradient magnetic

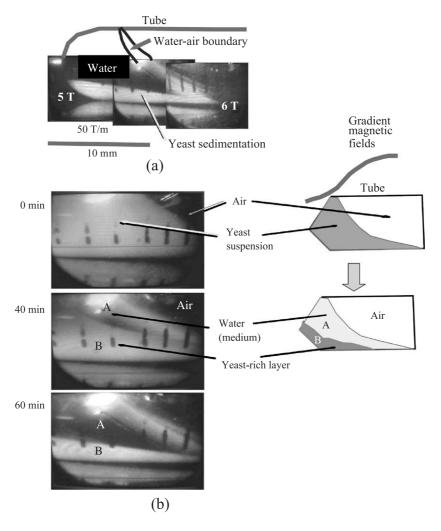


Fig. 7. Effects of magnetic fields on sedimentation of yeast in a peptone–glucose medium solution. (a) Boundary profiles of medium (water) between air and yeast sedimentation under magnetic fields of 5 to 6 T with a gradient of 50 T/m; (b) time course of the yeast sedimentation. 0 min: Homogeneously suspended yeast suspension formed an inclined surface at the upper-right of the photo. 40 min: A boundary between the medium solution (A) and the yeast-rich layer (B) appeared. 60-min: The yeast sedimentation exhibited a density gradient along the magnetic flux density gradients, which had a lower magnetic field at the left side of the photos.

fields are related to the volumetric magnetic susceptibility of the yeast. The observed changes in the yeast distribution in a gradient magnetic field resulted from differences between the volumetric magnetic susceptibility of the medium  $(K_A)$  and the yeast  $(K_B)$ . The difference in altitude  $(\Delta h)$  between the two points  $(x_1, x_2)$  of the boundary between the yeast sedimentation and the medium is expressed by

$$\Delta h = h(x2) - h(x1)$$
  
=  $(K_A - K_B)(B(x2)^2 - B(x1)^2)/2(\rho_A - \rho_B)g.$  (1)

where B is magnetic flux density, g is gravitational constant, and  $K_A$ ,  $K_B$ ,  $\rho_A$ , and  $\rho_B$  are volumetric magnetic susceptibilities and densities for medium (A) and for yeast sedimentation (B) [10]. The declining boundaries also indicated that the combined diamagnetic and gravitational force was directed normal to the boundary surfaces and that the yeast was exposed to the highest hydrostatic forces in the system.

Fig. 7(b) shows the time course of yeast sedimentation during 1 h of gradient magnetic field exposure after placing a homogeneously dispersed yeast suspension in a 15-ml tube. The tube was set horizontally along both magnetic field gradients and the axis of the bore of the superconducting magnet.

## 3.3. Gas pressure measurements

One pair of yeast suspensions in a flask was connected to a differential manometer. The manometer monitored both gas pressures inside the flask in the presence and absence of magnetic fields (6 T, 60 T/m). At the beginning of culture at 30  $^{\circ}$ C, the gas pressures in the flask were balanced and the differential manometer showed zero mm H<sub>2</sub>O. During the first 10 h, the differential pressure gradually decreased, being accompanied by an intermittent increase by 2 mm

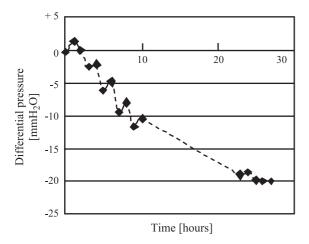


Fig. 8. Difference in gas pressures between yeast culture flask exposed and not exposed to the magnetic field. A time course of differential pressure is shown.

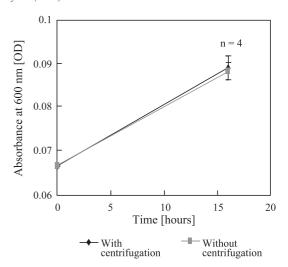


Fig. 9. Optical absorbance of yeast suspensions, which were incubated for 16 h with and without centrifugation at 1000 rpm.

 $H_2O$  (Fig. 8). After 1 day of culture, the differential pressure reached -20 mm  $H_2O$ , where the gas pressure inside the flask with the yeast suspension exposed to the magnetic field was less than the pressure of the control.

## 3.4. Centrifugal force and oxygen supply condition

Fig. 9 shows the optical absorbance of the yeast suspensions, which were incubated for 16 h with and without centrifugation. No distinct difference was observed between the two cases.

Fig. 10(a) shows the yeast proliferation monitored by the optical density at wavelength 600 nm which was affected by both the altitude of the culture medium solution and the size of the bottom area. The tube (15-ml centrifugal tube), which was set with its long axis parallel to the horizontal line, had much greater yeast growth, compared to the tube standing vertically.

In addition, Fig. 10(b) shows multiples of air supply conditions and tube angles. Yeast proliferation in the capped tube (closed tube) was compared with the tube with a parafilm covering (open tube), which has greater ventilation. In the control experiments (right columns), the absorbance of tube at 60° versus horizontal was lower than that of tube at 15°.

#### 4. Discussion

The results, which were presented in Figs. 3–6, indicated that the proliferation of the yeast in the gradient magnetic fields of up to 14 T was slow. After half a day of incubation, the optical density of the yeast suspension at wavelength 600 nm was different between those exposed and those not exposed to magnetic fields. The observed deceleration of yeast growth by magnetic fields was reproducible.

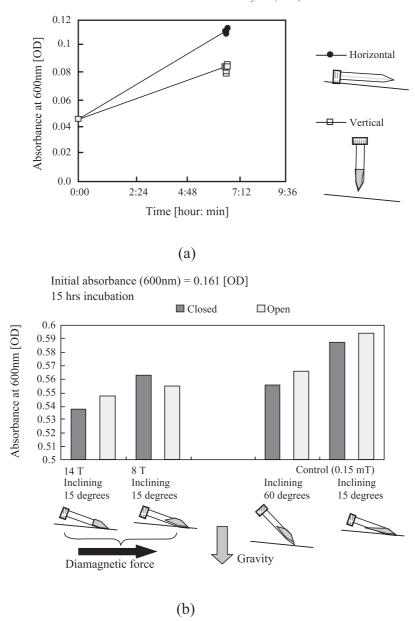


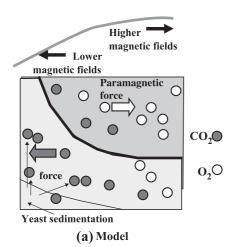
Fig. 10. Effects of tube setting configurations on yeast proliferation. (a) Effects of tube setting directions. Compared to each other, horizontal tubes and vertical tubes provided yeast with larger bottom areas and higher medium solutions, respectively. (b) Multiple air supply conditions and tube angles. Yeast proliferation in the capped tube (closed tube) was compared with that in the tube with a film covering (open tube), which has a greater degree of ventilation.

A comparison of gas pressures above the yeast suspension (Fig. 8) indicated that the gas pressure of the yeast culture atmosphere under magnetic fields was lower than that outside the magnetic fields. The experiments suggest the deceleration of  $CO_2$  gas production from yeast by strong magnetic fields on the order of 10 T.

We propose the following mechanisms as being responsible for the deceleration of yeast proliferation by magnetic fields.

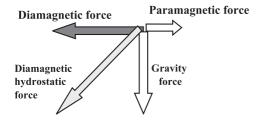
First, during yeast proliferation, the yeast consumed dissolved oxygen molecules in the suspension and generated carbon dioxide gas. The concentrations of paramagnetic oxygen molecules and diamagnetic carbon dioxide molecules changed during the yeast proliferation. The

total magnetism of the yeast culture system changed toward diamagnetism. It is considered that a concentration gradient of oxygen molecules occurs in the solution because the oxygen concentration beneath the air-solution boundary is higher than in the bottom part. When the gradient of magnetic susceptibility is in a vertical line, the magnetic field gradient in the horizontal direction caused a convectional flow (Fig. 11, mechanism-1). That induced convectional flow increased the oxygen concentration around the yeast, thereby enhancing the proliferation of the yeast. However, the obtained results indicated that the gradient magnetic fields decelerated the proliferation of the yeast.

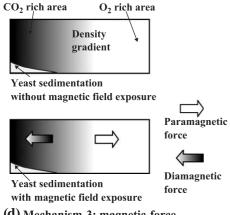


Magnetic susceptibility gradient +  $\frac{\pm}{0}$   $F = \pm \Delta \chi \ B \ db/dx$  Gradient of magnetic flux density  $\frac{\Delta \chi}{dB/dx}$ 

(b) Mechanism-1 : magnetic-force-induced convection



(c) Mechanism-2 : magnetic-force-enhanced hydrostatic force



(d) Mechanism-3: magnetic-force -modulated gas transport Another speculation is that a multiplication of hydrostatic forces and diamagnetic forces affected the proliferation of the yeast.

The gradient in the yeast sedimentation, which is shown in Fig. 7, suggests that the applied magnetic fields indirectly provided a force with the yeast via diamagnetic water. At the same time, the diamagnetic forces acted directly on the yeast and the yeast possibly drifted in the direction of the horizontal magnetic force.

A magnetic force acting on diamagnetic water molecules is converted into a hydrostatic force. The parting of water by magnetic fields changed the altitude of the water by 23 mm at 8 T [11] and 60 mm at 14 T, increasing the hydrostatic forces in the water. Due to the additional hydrostatic forces, the water pressure increased ( $\Delta p = 0.03$  to 0.1 N/cm<sup>2</sup>). In addition, the yeast conversion of paramagnetic oxygen to diamagnetic carbon dioxide gases decreased the paramagnetic forces of the oxygen, which directed toward higher magnetic fields (Fig. 11).

Fermentation by exponentially proliferating yeast generated the high pressure of the  $CO_2$  gas, and the diamagnetic force acting on the condensed  $CO_2$  gas multiplied the hydrostatic forces on the yeast sedimentation, as is shown in Fig. 11(c) (mechanism-2).

This speculation gave us the idea that the second possible mechanism for the observed delay in the proliferation of the yeast was due to magneto-hydrostatic forces of the diamagnetic molecules. An experiment with centrifugation in the absence of magnetic fields was conducted to check this second hypothesis. However, the results (Fig. 9) indicated that centrifugal forces of 1000 rpm had no effect on the proliferation of the yeast. The hydrostatic pressure of the centrifugation was estimated to be 3.7 N/cm<sup>2</sup> at the bottom of centrifugal tube. No distinct effect of centrifugal force was observed although the generated centrifugal forces were 30 to 100 times greater than the hydrostatic force.

The two mechanisms mentioned above did not support the effects of magnetic fields ( $\sim$  14 T) on the proliferation of yeast, which was suggested by an optical analysis in the study. We propose a third possible mechanism that depends

Fig. 11. Model and possible mechanisms for magnetic field effects on a yeast culture system. (a) Paramagnetic force acting on paramagnetic oxygen molecules directs toward the higher magnetic field, while diamagnetic forces acting on water, carbon dioxide, and yeast directs toward the lower magnetic fields (left side of figure). (b) Mechanism-1: The vertical gradient of magnetic susceptibility and the horizontal magnetic field gradient possibly enhance a convectional flow in the medium solution. (c) Mechanism-2: The diamagnetic forces and gravity force produce a diamagnetic—hydrostatic force. Fermentation by exponentially proliferating yeast decreases the oxygen concentration and increases the pressure of CO<sub>2</sub> gas, modulating the hydrostatic forces on yeast sedimentation. (d) Mechanism-3: Gradient magnetic force acting on the O<sub>2</sub>-CO<sub>2</sub>-H<sub>2</sub>O system with a concentration inhomogeneity disturbs gas-transport processes such as oxygen gas supply (from the right side to left sided of the yeast sedimentation in the bottom row) and CO<sub>2</sub> gas dispersion.

on the behavior of paramagnetic oxygen molecules under a spatial gradient magnetic field (Fig. 11, mechanism-3), and that is based on the same principle as the previously proposed magnetically induced convection. The present mechanism concerns the effect of gradient magnetic fields on dynamic behaviors, particularly the transport of gaseous materials [12–15]. Paramagnetic materials, such as oxygen molecules, and diamagnetic molecules, such as  $\rm CO_2$ , receive a magnetic force directing toward higher and lower magnetic fields, respectively. The gas-transport processes of  $\rm O_2$  and  $\rm CO_2$  are affected by gradient magnetic forces, and the oxygen supply for yeast differs from the oxygen supply outside the magnetic fields.

The gradient magnetic force acts on oxygen molecules [12-14]. However, the gradient magnetic fields of a superconducting magnet on the order of 10 T have negligible effects on  $O_2$  distribution in the equilibrium of dissolved oxygen concentration under the air with a constant partial pressure of oxygen gas [13,14].

In the experiments shown in Fig. 10(a), the solution in the parallel tube had a larger space than it did in the standing tube for yeast sedimentation, and the sedimenting yeast was closer to the air. The larger bottom area provided a lower yeast density at the bottom and enhanced both the diffusion of the metabolite and  $O_2$ – $CO_2$  gas exchange around the yeast. The altitude of the medium solution may have had an effect on the  $O_2$ – $CO_2$  gas exchange.

In the study testing the effect of oxygen permeability (Fig. 10(b)), the open tube provided an enhancement in yeast proliferation compared to the closed tube, because the O<sub>2</sub> gas was continuously supplied from outside the tube. Both the magnetic field exposures at 8–14 T and the standing culture tubes had a similar effect—cell growth deceleration—for the yeast culture in the centrifugal tubes. In the closed tube at 8 T, the absorbance of the suspension increased compared to that of the open tube. This was probably caused by an enhancement of convection and subsequent oxygen enrichment in the suspension, as shown in Fig. 11, mechanism-1.

The results in Fig. 10 suggest that the strong association of magnetic field exposures with the conditions of both the oxygen supply and the yeast suspension container. It was speculated that the yeast in the horizontal tubes had a much greater supply of oxygen, and thus proliferated faster.

In the centrifugal experiments, shown in Fig. 10, the centrifugal tube with 2 ml of solution was nearly filled to 100%. The solution had no contact with the air and, as a result, the oxygen supply had little effect. In addition, it is expected that both parameters, depth of medium solution and distribution of sediment, have no distinct effect on the oxygen gas transfer process when the container has no supply of additional air.

The most appropriate mechanism for the deceleration of yeast proliferation by magnetic fields is summarized as follows. The yeast was localized in the lower magnetic fields, as shown in Fig. 7, where diamagnetic gases such as carbon

dioxide gathered, and the gradient magnetic force acting on the O<sub>2</sub>-CO<sub>2</sub>-H<sub>2</sub>O system with a concentration inhomogeneity disturbed the transport of paramagnetic oxygen gases from higher magnetic fields to lower magnetic fields.

Recently, we reported on the effect of static magnetic fields of up to 14 T on gene expressions in yeast. A respiratory gene was up-regulated under exposure to magnetic fields of 14 T while no effect was observed under fields of 5 T [16].

Future studies are needed in order to clarify the relationships between the effects of magnetic fields on the proliferation of yeast and on the respiratory response of yeast to magnetically modulated  $O_2$  and  $CO_2$  gas pressures.

In conclusion, yeast in a liquid—gas mixture system under gradient magnetic fields (14 T) exhibited decelerated growth. The spatial gradient magnetic fields whose magnetic flux densities *B* were 5–14 T with a maximum gradient of 94 T/m, provided magnetic forces on paramagnetic oxygen gas and on diamagnetic materials (carbon dioxide, water, etc.) and affect the spatial distribution and/or transfer processes of elements. In this report, a decrease in oxygen supply was probably responsible for the observed deceleration in yeast growth.

We expect that the mechanism, modulation of oxygen supply by a gradient magnetic field, can also provide condition where the oxygen supply for yeast is accelerated. The condition requires that the yeast be located in points of maximum magnetic flux density. For example, a culture medium solution whose columetric diamagnetic susceptibility is larger than yeast will exhibit this condition.

## Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, and by Transport Technology Research from the Corporation for Advanced Transport and Technology.

## References

- E. Beaugnon, R. Tournier, Levitation of organic materials, Nature 349 (1991) 470.
- [2] S. Ueno, M. Iwasaka, Properties of diamagnetic fluid in high gradient magnetic fields, J. Appl. Phys. 75 (1994) 7177-7179.
- [3] N. Hirota, T. Homma, H. Sugawara, K. Kitazawa, M. Iwasaka, S. Ueno, H. Yokoi, Y. Kakudate, S. Fujiwara, M. Kawamura, Rise and fall of surface level water solutions under high magnetic field, J. Appl. Phys. 34 (1995) L991–L993.
- [4] J. Torbet, J.M. Freyssinet, G. Hudry-Clergeon, Oriented fibrin gels formed by polymerization in strong magnetic fields, Nature 289 (5793) (1981) 91–93.
- [5] Y. Ikezoe, N. Hirota, J. Nakagawa, K. Kitazawa, Making water levitate, Nature 393 (1998) 749-750.
- [6] M. Iwasaka, S. Ueno, Optical measurements of magnetophoresis of macromolecules, IEEE Trans. Magn. 34 (1998) 2129–2131.
- [7] H. Iwahashi, S. Fujii, K. Obuchi, S.C. Kaul, A. Sato, Y. Komatsu,

- Hydrostatic pressure is like high temperature and oxidative stress in the damage it causes to yeast, FEMS Microbiol. Lett. 108 (1993) 53-57.
- [8] E. Palou, A. Lopez-Malo, G.V. Barbosa-Canovas, J. Welti-Chanes, P.M. Davidson, B.G. Swanson, High hydrostatic pressure come-up time and yeast viability, J. Food Prot. 61 (1998) 1657–1660.
- [9] F. Abe, K. Horikoshi, Hydrostatic pressure promotes the acidification of vacuoles in *Saccharomyces cerevisiae*, FEMS Microbiol. Lett. 130 (1995) 307–312.
- [10] H. Sugawara, N. Hirota, T. Homma, M. Ohta, K. Kitazawa, H. Yokoi, Y. Kakudate, S. Fujiwara, M. Kawamura, S. Ueno, M. Iwasaka, Magnetic field effect on interface profile between immiscible nonmagnetic liquids—enhanced Moses effect, J. Appl. Phys. 79 (1996) 4721–4723.
- [11] S. Ueno, M. Iwasaka, Parting of water by magnetic fields, IEEE Trans. Magn. 30 (1994) 4698-4700.

- [12] S. Ueno, K. Harada, Redistribution of dissolved oxygen concentration under strong DC magnetic fields, IEEE Trans. Magn. 18 (1982) 1704–1706.
- [13] S. Ueno, M. Iwasaka, T. Kitajima, Redistribution of dissolved oxygen concentration under magnetic fields up to 8 T, J. Appl. Phys. (1994) 7174–7176.
- [14] S. Ueno, M. Iwasaka, G. Furukawa, Dynamic behavior of dissolved oxygen under magnetic fields, IEEE Trans. Magn. 31 (1995) 4259–4261.
- [15] N. Hirota, Y. Ikezoe, H. Uetake, J. Nakagawa, K. Kitazawa, Magnetic field effect on the kinetics of oxygen dissolution into water, Mater. Trans. JIM (2000) 976–980.
- [16] M. Ikehata, M. Iwasaka, J. Miyakoshi, S. Ueno, Effects of intense magnetic fields on sedimentation pattern and gene expression profile in budding yeast, J. Appl. Phys. 93 (2003) 6724–6726.