# Yeast cells proliferation on various strong static magnetic fields and temperatures

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**Abstract.** The effect of strong magnetic fields on activities of yeast cells were investigated. Experimental yeast cells were cultured in 5 ml of YPD(Yeast extract Peptone Dextrose) for the number density of yeast cells of $5.0 \pm 0.2 \times 10^6$/ml with various temperatures and magnetic fields up to 10 T. Since the yeast cells were placed in the center of the superconducting magnet, the effect of magnetic force due to the diamagnetism and magnetic gradient was negligibly small. The yeast suspension was opened to air and cultured in shaking condition. The number of yeast cells in the yeast suspension was counted by a counting plate with an optical microscope, and the time dependence of the number density of yeast cells was measured. The time dependence of the number density of yeast cells, $\rho$, of initial part is analyzed in terms of Malthus equation as given by $\rho = \rho_0 \exp(kt)$, where $k$ is the growth coefficient. It is found that, the growth coefficient under the magnetic field is suppressed compared with the control. The growth coefficient decreasing as increasing magnetic field and is saturated at about 5 T. On the other hand, it is found that the suppression of growth of yeast cells by the magnetic field is diminished at high temperatures.

## 1. Introduction

The relation between magnetic field and living organism has been investigated by many groups. It was reported that low-frequency magnetic fields such as 50 Hz and 10 mT affected yeast *Saccharomyces cerevisiae* activities: the the number of yeast cells were decreasing and their growths were restricted[1]. Many reports are cited in Ref. [1] and the effect of the environment magnetic field is discussed.

On the other hand, no effect on the growth of yeast *Saccharomyces cerevisiae* was reported in the AC magnetic field of 50 Hz, 2.45 mT[2]. The effect of AC magnetic field is difficult to detect, since the joule heat is always occurred in measurements and it causes the increase of the temperature.

Recent development of cryocooler allows us to use superconducting technology easier, and strong magnetic field over 10 T in room temperature space can be applied by superconducting magnet[3]. This new technology accelerated many applications of such as physics, chemistry and biology fields. For example, the growth of *Escherichia coli* in magnetic field of 5.2–6.1 T is reported and the death is suppressed 100,000 times than that under a geomagnetic field[4]. This result suggests that the growth rate of *Escherichia coli* is suppressed by magnetic field. In addition, it is reported that strong static magnetic fields (5 T or 9 T) induce mutations through elevated production of intracellular superoxide radicals in *Escherichia Coli*[5].

In the case of effect of static magnetic field on yeast cell activities, Ikehata *et al.* found that the sedimentation pattern of yeast cells at 5 T was changed although the static magnetic field did not affect the expression level of any gene in yeast cells. Iwasaka *et al.* performed the proliferation of yeast cells under the gradient magnetic field at 9–14 T. They found the proliferation of the yeast cells was suppressed and they tried to explain the observed phenomenon by magnetic force by the diamagnetism of oxygen and the gradient magnetic field.

It is desire to investigate the pure effect of the magnetic field to yeast cell without that of the induced environmental changes by the magnetic field. In the present study, yeast cells were proliferated in strong static magnetic field up to 10 T at various temperatures with small effect of the magnetic-induced force. The effect of the static magnetic field is reported and the possible effects of magnetic field are discussed.

## 2. Experimental

### *2.1. Japanese Sake mash*

First, we started to study what sort of difference in taste would arise when a magnetic field was applied to yeast cells while brewing Japanese sake. Since sake is an extremely delicate brew, there is the potential for major differences to arise. Furthermore, sensory evaluation (sake tasting) by the human is very sensitive and thus able to detect even subtle differences.

Since the sake manufacturing process is rather complex, some Koji (aspergillus oryzae, the mold used in the brewing process) extract was prepared and then some sake mash was provided. Koji extract is made by rice and malted rice that has been mixed together and saccharified at 55°C for 4 days. Koji extract has an amber color and is sweet tasting. The concentration of the glucose was 15%. Then sake yeast K-7 was put for making refined sake directly into it. The number density of the yeast cells was $2.5 \pm 0.1 \times 10^6$/ml. Two 100 ml bottles of sake mash were prepared. The bottles were sealed. Yeast cell converts glucose into alcohol through fermentation. The temperature of the Japanese sake mash was maintained at 15°C and a magnetic field of 10 T was applied. The other controlled sake mash was also prepared without a magnetic field for comparison. Since the cultivation is stationary and the low temperature is kept, the cultivation needs about one week.

The static magnetic field was applied by a superconducting magnet cooled by cryocooler manufactured by Japan Superconductor Technology Inc.(JASTEC). Maximum magnetic field is 10 T. The induced magnetic field is uniform within 1.4% in a sphere with radius of 40 mm. Temperature control of the yeast suspensions is very important factor in the present experiment. Therefore, two jacketed vessels were prepared. One vessel was put into the room temperature bore of the superconducting magnet system, and the other vessel was for control. The jacket water were circulated to two vessels in series and the temperature of the water was controlled by a chiller unit. The fluctuation of the temperature was ±0.1°C. The sake mash bottle was put into the water in the vessel.

The taste test was performed by alcoholic beverage appraisers from the Fukuoka Regional Taxation Bureau. In addition, low-boiling flavor components and organic acid components were measured. The number density of the yeast cells $\rho$ in the sake mash for magnetic field $\rho_{\mathrm{M}}$ and for control $\rho_{\mathrm{C}}$ were also counted by the standard plate count technique with an optical microscope and a counting plate.

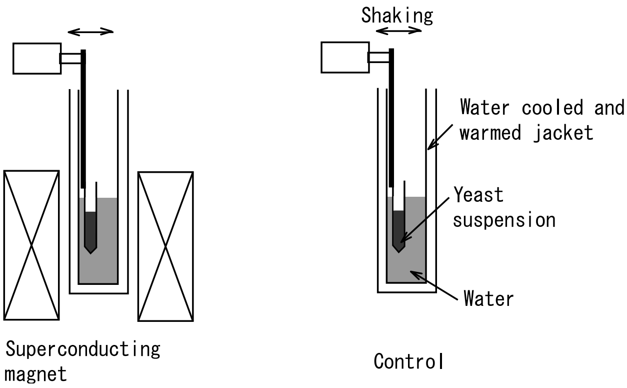


**Figure 1.** Configuration of sample holder.

*2.2. Experimental yeast*

The second experiment was performed for experimental yeast. The experimental time was one week in previous sake mash experiment, since the temperature was low and the condition was the stationary cultivation. Therefore, in the second experiment the temperature was controlled to be higher and shake cultivation was used. The yeast used in the second experiment was so-called experimental yeast W303-1a. And the yeast cells were pre-cultured on a YPD (Yeast extract Peptone Dextrose) agar plate. Then it was moved to YPD suspension and 12 hours incubation was performed in a water bath with shaking. Then, the pre-cultured yeast cells were put to 10 ml YPD with the number density of yeast cells of $5.0 \pm 0.2 \times 10^6$/ml. This yeast suspension is divided into two tubes. Here, YPD was composed by Yeast extract: Peptone: Dextrose=5 g: 10 g: 10 g to 500 ml RO water.

The magnetic field was applied by the superconducting magnet up to 10 T. The temperature was controlled by the jacketed vessels in the range of 20–30°C. The tubes of the yeast suspensions were put inside the jacketed vessels as shown in figure 1. The tubes were shaken about 1 Hz to maintain a good condition for the proliferation. The shake stroke was 10 mm resulting in small magnetic-induced force, because the magnetic field is uniform within 1.4% in a sphere with radius of 40 mm. In the shaken condition, since the tubes were open to expose the yeast suspension to the air, oxygen was enough supplied to the yeast cells, and the activity of the yeast cells was accelerated. The position of the yeast suspension was the center of the superconducting magnet resulting in very small effect of the magnetic-induced force by the diamagnetism of oxygen or water and magnetic gradient. And the magnetic field was applied in the range of 0$\sim$ 10 T.

The time dependence of the number density of the yeast cells $\rho$ in the suspension was measured. The number density of the yeast cells for magnetic field $\rho_{\mathrm{M}}$ and for control $\rho_{\mathrm{C}}$ were counted by standard plate count technique.

## 3. Results and Discussion

The result of quantitative analysis for chemical components after about one week alcohol fermentation of Japanese sake mash in the magnetic field of 10 T is listed in Table 1. The differences of samples of magnetic field and control in (a) low-boiling flavor component and (b) organic acid are within 1.6ppm except pyruvic acid. Hence, the effects of magnetic field to flavor component and organic acid are very small. However, the concentration of the glucose in the magnetic field is larger than that in the control, and the concentration of the ethanol in the magnetic field is smaller than that in the control. These results are also confirmed by the organoleptic examination by the members of Fukuoka Regional Taxation Bureau, i.e., three appraisers concluded sweet taste for the sample in the magnetic field. Figure 2 shows the time dependence of the number density of the yeast cells at 10 T and 15°C. It is found that the number density of the yeast cells in the magnetic field, $\rho_{\mathrm{M}}$, is suppressed than the case of the

**Table 1.** Results of quantitative analysis for (a) low-boiling flavor component, (b) organic acid, and (c) glucose and ethanol in the magnetic field and control.

| (a) low-boiling flavor component | | | | |
|---|---|---|---|---|
| | isobutyl alcohol | isoamyl alcohol | isoamyl acetate | ethyl caproate |
| magnetic field(ppm) | 10.4 | 32.7 | 0.7 | 0.9 |
| control(ppm) | 9.7 | 34.2 | 0.7 | 1.0 |

| (b) organic acid | | | | | | |
|---|---|---|---|---|---|---|
| | succinic acid | malic acid | lactic acid | pyruvic acid | citric acid | acetic acid |
| magnetic field(ppm) | 73.5 | 86.8 | 30.7 | 668.3 | 104.0 | 85.9 |
| control(ppm) | 71.9 | 87.8 | 30.3 | 648.4 | 104.8 | 87.5 |

| (c) | glucose | ethanol |
|---|---|---|
| magnetic field(%) | 8.1 | 3.5 |
| control(%) | 7.9 | 3.7 |

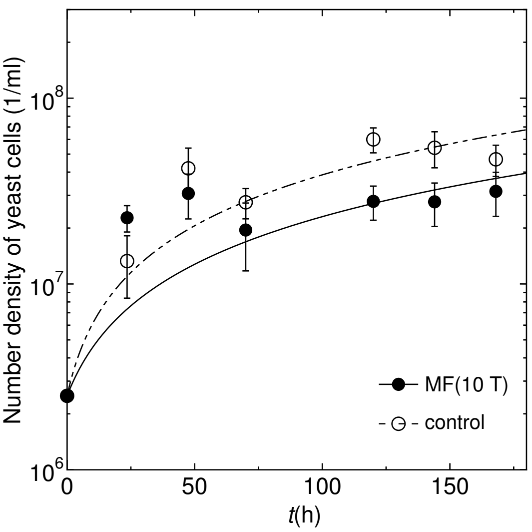


**Figure 2.** Time dependence of number density of yeast K-7 cells at 10 T and 15°C.

control, $\rho_C$. These results suggests that the activity of the yeast cells is suppressed by the strong static magnetic field resulting in the small number density of the yeast cells. That is, the glucose is not sufficiently converted to the ethanol in the magnetic field due to the low activity of yeast cells, resulting in sweet taste.

The control of the activity of the yeast cells is mainly done by the control of the temperature in brewing. For example, high quality sake is made in low temperature such as 10–15°C and the fermentation rate is controlled to be lower than usual sake brewing, and particular flavor is produced. If the fermentation rate is controlled by the magnetic field, new kinds of taste could be created in new circumstance with the magnetic field. For example, much slower fermentation could be achieved at low temperature and strong magnetic field. Therefore, it should be confirmed that the effect of the magnetic field to the yeast cells in various magnetic fields and temperatures. In this study, the experimental yeast W303-1a is used for the measurement of the time dependence of the number density of the yeast cells in shirking condition in various conditions.

Figure 3 shows the result of the time dependence of the number density of the experimental yeast W303-1a cells at 10 T and 20°C for shaking condition as shown in figure 1. The number density of the yeast cells is almost saturated at 30 hours, since the nutrition in YPD is limited.

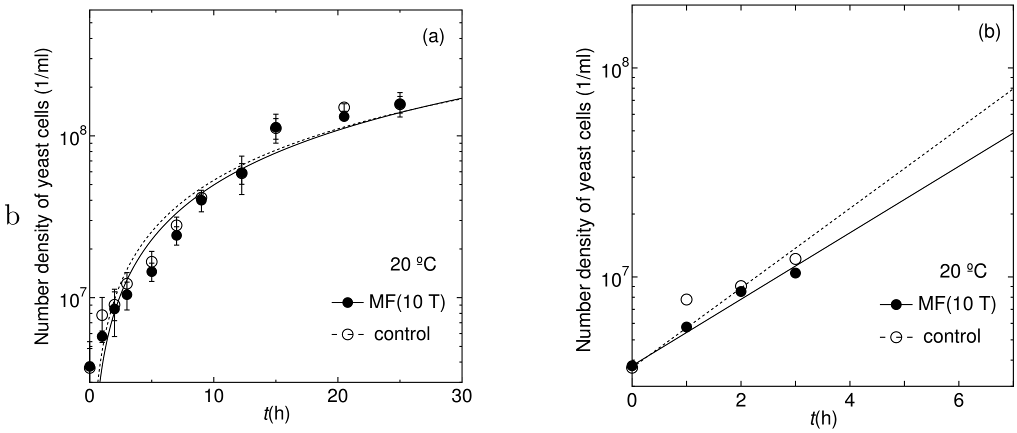


**Figure 3.** Time dependence of number density of yeast cells at 10 T and 20°C for yeast W303-1a.

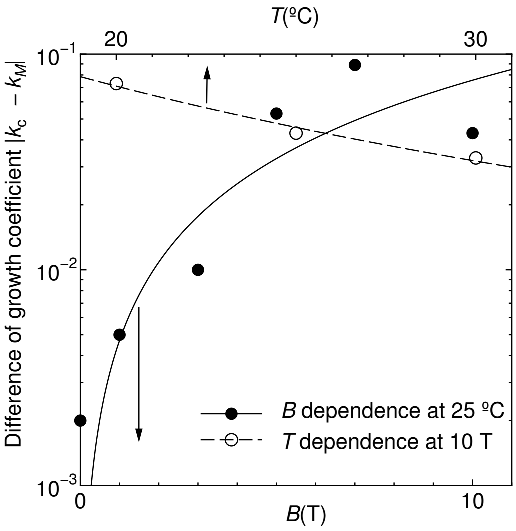


**Figure 4.** Magnetic field dependence of growth coefficient (solid line) at 25°C and temperature dependence of growth coefficient (dashed line) at 10 T.

The rate of the fermentation of 30 hours is faster than the previous experiment of Japanese sake mash of 7 days, since the temperature is high and there exists much oxygen provided by the shaking condition. It is found that the number density of the yeast cells in the magnetic field is suppressed in the time less than 10 hours. This period is known as most active time in the proliferation of the yeast cells. These results are confirmed in the temperature range of 20–30°C and the magnetic field range of 1–10 T. Reproducibility tests are performed at 25°C and 5 T and almost the same results are obtained.

The rate of the proliferation is tried to be defined in the most active time less than 6 hours. The time dependence of the number density of the yeast cells, $\rho$, is analyzed in terms of Malthus equation as given by

$$\rho = \rho_0 \exp(kt). \tag{1}$$

In the above, $k$ is the growth coefficient and $\rho_0$ is a constant indicating the initial number density of the yeast cells at initial time $t = 0$. The example of determination of $k$ is shown in figure 3(b). It is found that the growth coefficients $k$ of two cases shown as the slopes in the figure are different and the $k$ for the case of the magnetic field $k_{\mathrm{M}}$ is smaller than that of the control $k_{\mathrm{C}}$. The difference of the growth coefficient $|k_{\mathrm{C}} - k_{\mathrm{M}}|$ of the magnetic field, $k_{\mathrm{M}}$, and the control, $k_C$ is shown as functions of magnetic field and temperature in figure 4. The temperature is 25°C for the magnetic field dependence, and the magnetic field is 10 T for the temperature dependence. The difference of the growth coefficients becomes small as increasing temperature, since the activity of the yeast cells is high at higher temperatures and the effect of the magnetic field is less remarkable. It is also found that the effect of magnetic field for the growth coefficient increases as increasing the magnetic field. However, it seems to be saturated around 5 T. Therefore, it is necessary to confirm the effect of the magnetic field on the yeast

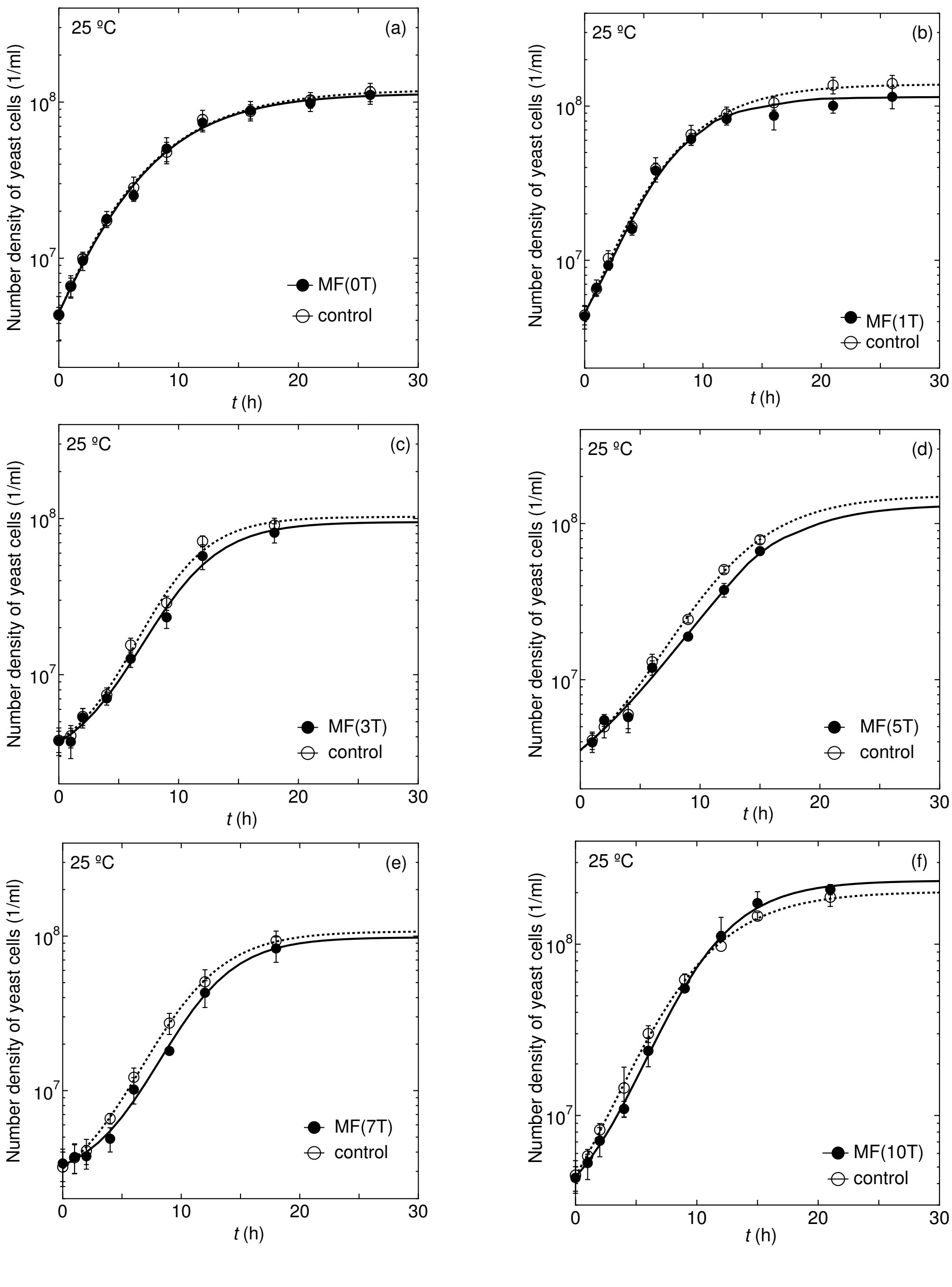


**Figure 5.** Time dependence of number density of yeast cells for various magnetic fields at 25°C, (a) 0 T, (b) 1 T, (c) 3 T, (d) 5 T, (e) 7 T, (f) 10 T.

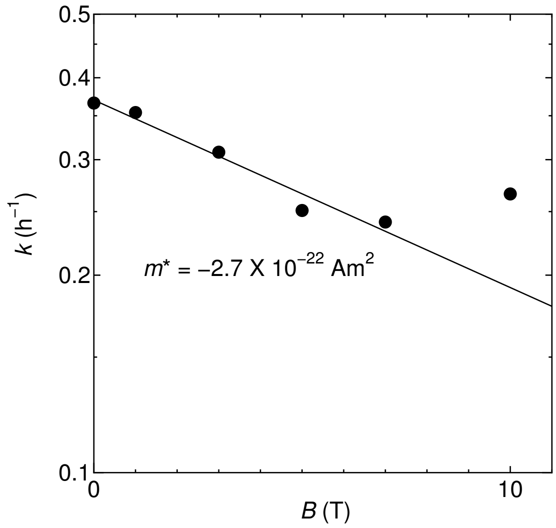


**Figure 6.** Magnetic field dependence of the growth coefficient, $k$ at 25°C.

cells activities at stronger magnetic fields higher than 10 T. The time dependence of the number density of the yeast cells at 25°C with various magnetic field are shown in figure 5. The difference is enough small at 0 T, indicating the lower limit of detecting the difference of $k$ in the present experiment. The difference of the growth coefficient becomes large as increasing magnetic field and saturates around 5 T as abovementioned in figure 4.

Here, the growth coefficient is discussed by using classical Arrhenius type equation which is frequently used in physicochemical investigation. The magnetic field dependence of the growth coefficient is described by

$$k(B) = k_0 \exp\left(\frac{m^*}{k_{\rm B}T}B\right), \tag{2}$$

where $k_{\rm B}$ is the Boltzmann constant and $m^*$ is the magnetic growth activation moment which represent the sensitivity to magnetic field[8]. That is, large value of m* means that the growth activation of yeast cells is sensitive by the magnetic field. Figure 6 shows the semi-logarithmically plot of magnetic field dependence of the growth coefficient. Hence, the magnetic growth activation moment is determined from the initial slope as $m^* = -2.7 \times 10^{-22}$ Am$^2$. It is desired to measure $m^*$ for various cases such as various temperatures or various kinds of yeast cells.

It is difficult to know the mechanism for the strong static magnetic field effect of the yeast cells from the present experiment. However it is interesting to know that the effect of the magnetic field is not related to the joule heat since the magnetic field is static, and is not related to the magnetic-induced force by water or oxygen since the magnetic gradient is negligibly small in the present study. It should be noted that the effect of the oxygen is small in the experiment with the experimental yeast, since it is shaking condition and this physical effect is much larger than the magnetic-induced force to the oxygen. It is reported that the vaporization of water is enhanced by gradient magnetic field more than 10 T$^2$/m[9]. However, this effect seems to be small since the tubes is vertically placed in the center of the superconducting magnet and the gradient magnetic field is small. Therefore, it is desired to investigate the essential explanation of the magnetic field on the activities of the yeast cells.

## 4. Summary

In this study, the effect of strong static magnetic field to the activities of the yeast cells were measured in various magnetic fields and temperatures. As a result, the proliferation of the yeast cells is suppressed by applying the static magnetic field. Therefore, the taste of sake mash becomes sweet when magnetic field is applied during the fermentation process. The effect of the magnetic field is saturated around 5 T. When the temperature is high, the effect of the magnetic field becomes small, since the proliferation activity is high at high temperatures. It is considered that the control of the fermentation of the yeast cells is possible by the strength of the magnetic field in addition of the control of the temperature. Therefore, the new taste of alcoholic beverage is expected with applying static magnetic field. For example, much slower fermentation could be achieved at low temperature with strong magnetic field.


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