A comparison of the fermentation performance and stress tolerance of baker's yeast cells grown in media with or without magnesium addition

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Abstract

Bioethanol is an alternative fuel that was developed to overcome the depletion of fossil fuels. It is produced by converting sugars to ethanol through fermentation using microorganism activity, especially the yeast Saccharomyces cerevisiae. However, during ethanol fermentation, the yeast cells are exposed to various environmental stress factors which can reduce their performance. Some metal ions are known to have an impact on yeast cell's tolerance against environmental stress factors. The present study investigates the role of magnesium ions in the fermentation performance and stress tolerance of a baker's yeast strain.

Yeast cells were grown in media with or without magnesium ions. Growth and fermentation kinetic parameters were then monitored every 6 hours during the first 24 hours followed by 12 hours intervals for the rest fermentation. After 24 hours of fermentation, stress tolerance assays were performed against ethanol, hyperosmotic, oxidative and acetic acid stress. The results of the present study indicate that magnesium ions can enhance the fermentation performance of yeast cells as indicated by better specific growth rates, substrate utilization, ethanol productivity and ethanol yield. Magnesium also showed a protective effect against all stress factors tested in this study.

Keywords: Bioethanol, *Saccharomyces cerevisiae*, magnesium, stress tolerance.

Introduction

Bioethanol is produced by fermenting sugary, starchy, or cellulosic material using microorganisms. The utilization of bioethanol can reduce fossil fuel requirements[1]. Bioethanol is renowned as an environmentally friendly and renewable alternative energy source[2]. When bioethanol is used as a fuel, the net amount of CO_2 in the atmosphere will stay the same because it has a closed carbon cycle[3]. Compared to the other ethanol producing microorganisms, *S. cerevisiae* is better[4]. It has a broad substrate preference and the waste from the fermentation process can be utilized as animal feed[4].

When used in the ethanol fermentation process, yeast cells may be exposed to various environmental stresses such as high ethanol concentrations, nutrient starvation and heat shock. These can affect cell activity and decrease ethanol production[5]. When yeast cells are exposed to a stress factor, it will be detected by the yeast cells and signal transduction pathways to change gene expression and metabolism will be induced. Consequently, the cell will be able to repair the damage caused by stress[6]. These protective mechanisms can be enhanced by genetic modification, growth medium modification, or a combination of both[7].

Previous studies have showed that the adverse effects of stress conditions on yeast cells can be overcome by supplementing metal into their growth media[8]. Metal ions, especially divalent ions, play an important role by activating certain glycolytic enzymes. When cells experience a deficiency in metal ions, their fermentation performance will not be optimal and ethanol production may be decreased[9].

As a macronutrient for yeast cells, magnesium is involved in many physiological and biological functions such as cell division and growth, activation of enzymes and stress protection against environmental stress[10]. According to Ismail et al[11], magnesium supplementation into the growth media can enhance both xylose consumption and ethanol production under 30 mM of acetic acid stress. The optimum concentration of magnesium was found to be 45 ppm. Birch and Walker[12] showed that magnesium has a protective effect on yeast cells from heat shock (42°C) and ethanol shock (10% v/v). In the presence of heat shock or ethanol shock, there were more viable cells grown in the media with magnesium supplementation than in the media without magnesium supplementation.

This study was conducted to investigate the effects of magnesium supplementation on the fermentation performance and stress tolerance of baker's yeast in defined fermentation media. Stress conditions used in the present study were high ethanol concentrations, acetic acid, hyperosmotic and oxidative stresses.

Material and Methods

Yeast strain and maintenance: *S. cerevisiae* strain A12 was selected for the present study. It was selected because previous studies indicate that it can produce a high concentration of ethanol[13]. The yeast strain was maintained on slopes of yeast extract peptone (YEP) media containing (w/v) 0.5% yeast extract, 0.5% bacteriological peptone, 0.3% potassium dihydrogen phosphate, 1% glucose and 1.5% agar. The slopes were stored at 4 ºC.

Growth media and culture conditions: The cells were grown in a defined fermentation media (yeast nitrogen based (YNB) broth) without magnesium ions containing 10% w/v glucose. Starter cultures were inoculated from the slopes and grown overnight (~16 hours) in a 180 orbital per minute (opm) orbital shaker at room temperature.

Experimental batch culture conditions and sampling: Aerobic cultures were prepared by aseptically adding YNB media to a sterile Erlenmeyer flask and inoculating these with overnight cultures to give an initial viable cell number of 10^6 cells/mL. The ratio of flask size to culture volume was maintained at a minimum of 4:1 to ensure adequate oxygen mixing. To investigate the effects of magnesium supplementation, magnesium was added to the experimental cultures in various concentrations as follows: 0 (control), 10, 100 and 1000 ppm. Samples from each culture were aseptically drawn off with a micro pipette every 6 hour for the first 24 hours and the rest was removed every 12 hours until 96 hours were reached. Examination of the samples included measuring the growth rate by measuring optical density, viable cell numbers, total cell numbers, percent of viability and glucose and ethanol concentrations.

Growth Rate: Yeast growth was monitored by measuring the optical density of the culture at 600 nm (OD_{600nm}) using a Spectronic Genesys 10 UV-Vis spectrophotometer, making dilutions where necessary.

Viable cell numbers: Viable cell numbers were assessed using the methylene violet staining method and light microscopy (400 × magnification) using a Neubauer-type haemocytometer. Methylene violet staining is proposed as a better method for monitoring yeast cell viability compared to the traditional methylene blue staining method[14].

Percent viable cell: When counting, both live and dead cells were recorded to obtain the total number of cells per mL. The number of viable cells were then divided by the total cells and multiplied by 100 to give the percentage of viable cells.

Glucose concentrations: Glucose concentrations were determined using the alkaline potassium ferricyanide method[15]. 0.2 mL of sample and 0.6 mL of potassium ferricyanide solutions were added to a tube and mixed thoroughly. The reaction mixture was heated at 100°C for 10 minutes and cooled to room temperature. Then, it was diluted with 4 mL of distilled water and mixed thoroughly. The absorbance was then recorded at 420 nm after setting the spectrophotometer to zero with distilled water as reagent blank[17]. The glucose standard solutions were made in concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL.

Ethanol concentrations: 25 µL of sample and 25 µL of NAD^+ solution were added to a tube containing 1.25 mL semicarbazide solution and mixed thoroughly. After being finely mixed, 5 µL of alcohol dehydrogenase solution (4000 unit/mL) was added and mixed thoroughly. The reaction mixture was then incubated at 30°C for 40 minutes. The absorbance was then read at 340 nm after setting the spectrophotometer to zero with the reagent blank[16]. The ethanol standard solutions were made in concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05% v/v.

Yeast cell stress tolerance assay: Cells were drawn from 24 hours cultures. YNB media containing 2% glucose were prepared and divided into four treatments. Each treatment had different stress factors i.e. 27% (w/v) sorbitol (hyperosmotic stress), 67 mM acetic acid (weak acid stress), 4 mM hydrogen peroxide (oxidative stress) and 7% (v/v) ethanol (ethanol stress). To investigate the effects of magnesium, one pair of the experimental sample, both with and without magnesium addition, was set for each stress condition. The cell culture was diluted until ~0.1 OD was reached and this was designated as 0 h. The cells were then grown at room temperature with 180 opm of shaking speeds for 24 hours. The OD value at 600 nm was monitored and viable cells were calculated as a representation of OD values from control media without stress conditions[17].

Statistical analysis: The raw data was compiled into Minitab 15® for Windows®. This software package was then used to perform statistical analysis. When significant ($p < 0.05$) differences were detected in the one-way ANOVA, the test was followed by the Tukey post hoc test to determine which data differed significantly.

Results and Discussion

Kinetics and Performance of Fermentation: The growth parameters of baker's yeast cell grown in media with and without magnesium are presented in figure 1. Both OD_{600nm} (figure 1A) and total cell numbers (figure 1B) indicate that cells grown in supplemented media have significantly higher cell numbers compared to cells that are grown in media without supplementation. There are no differences between cells grown in different levels of magnesium addition. Exponential phase growth was observed during the first 24 hours. The maximum specific growth rate in the exponential phase of yeasts grown with magnesium was significantly higher than in those grown without magnesium (table 1).

The total viable cell number (figure 1C) and cell viability (figure 1D) showed a similar trend as the growth curve, as the cells grown in magnesium supplemented media were significantly more compared to the control media. After the exponential growth phase, the viability of control yeast cells decreased dramatically whereas the viability of cells grown in supplemented media decreased slower. Recent studies show that magnesium supplementation can enhance cell growth and viability.

According to Walker[18], magnesium can enhance cell viability because it is involved in stimulating DNA synthesis that favor cell division. This finding is similar to another study by Trofimova et al[19] who found that magnesium can

stimulate cell growth and the maximum cell biomass was reached at 300 ppm of magnesium. These studies indicate that cell growth can be stimulated by magnesium.

Figures 2A and 2B show that cells grown in magnesium supplemented media have better sugar consumption and ethanol production capability. This is evident by the results at the end of fermentation as the supplemented media have lower residual glucose and higher ethanol production compared to the control media. Based on table of kinetics parameters of fermentation (table 1), the glucose consumption rate of yeast grown in media with 100 and 1000 ppm supplemented magnesium was significantly higher than the control. It was also found that 1000 ppm of magnesium ions was the optimum concentration to get a higher ethanol productivity and ethanol yield.

The present study indicates that magnesium can enhance the fermentation kinetics of baker's yeast. This result is similar to the study by Birch et al[20] who reported that increasing the concentration of magnesium from 50 to 800 ppm also enhanced glucose consumption and ethanol production rates.

Yeast Tolerance against Stress Conditions Magnesium enhanced yeast cell tolerance against the stress factors tested in the present study. When cells were grown under ethanol stress (figure 3A), the cells grown with 10 and 100 ppm magnesium had significantly higher growth compared to the control and 1000 ppm. The highest relative growth reached was ~4.5%. Under weak acid stress (figure 3B), 10 ppm magnesium gave the best protection as indicated by the higher relative growth compared to the control. Under hyperosmotic stress (figure 3C), cells grown in magnesium supplemented media showed a significant difference compared to control and the highest relative growth reached was ~80%.

Under oxidative stress (figure 3D), cells grown in magnesium supplemented media showed a significant difference compared to the control and the highest relative growth reached was ~85% with 100 ppm as the optimum concentration. The present study indicates that magnesium can enhance yeast tolerance. Furthermore, the baker's yeast strain used in the present study is very sensitive to ethanol and weak acid stress, as proved by the lower relative growth compared to cells grown under hyperosmotic and oxidative stress.

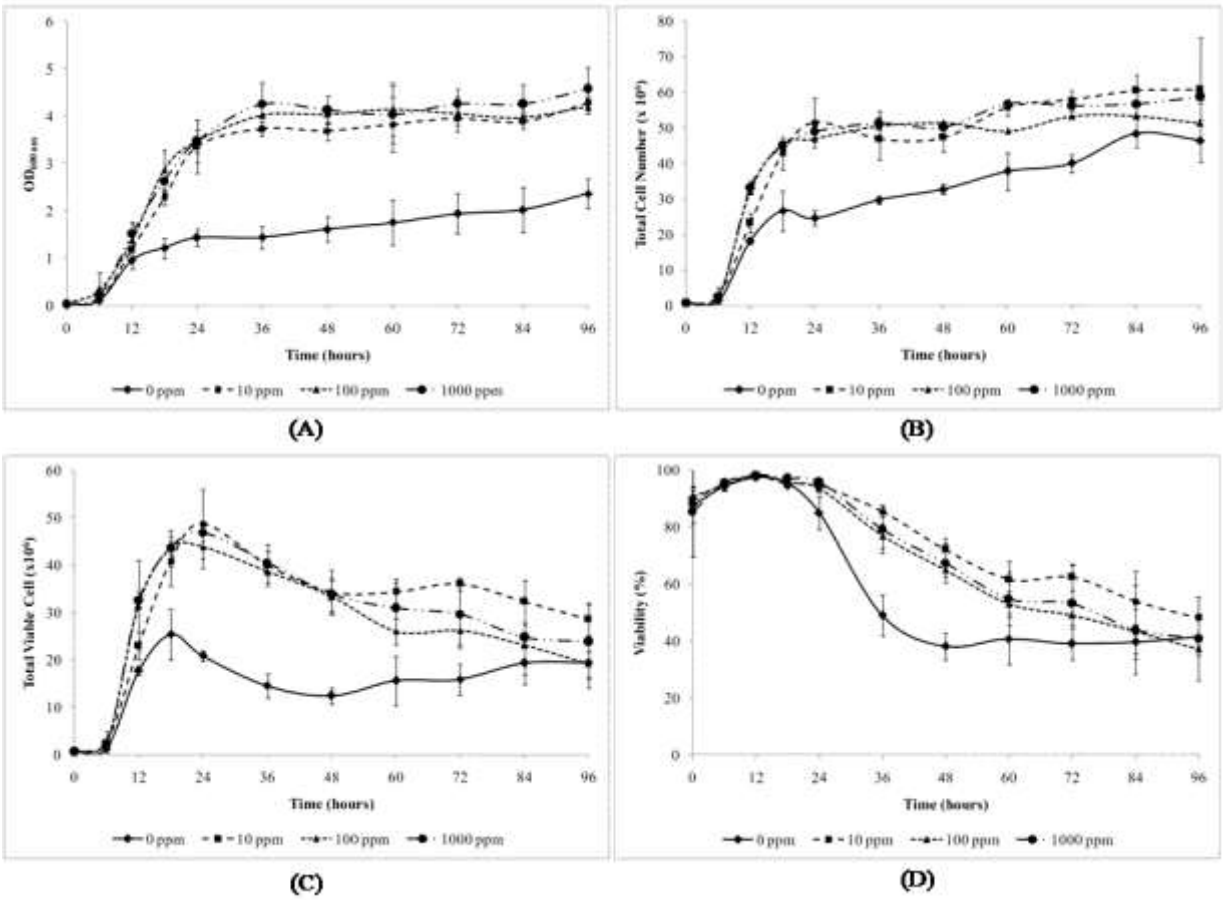

Figure 1: The growth parameters of baker's yeast cells grown in media with and without magnesium supplementation. (A) $OD_{600\ nm}$, (B) total cell numbers, (C) total viable cell number, (D) cell viability. The legends on the figures indicate the concentration of Mg^{2+}. Data presented are means of three replicates. Optical density was measured using a spectrophotometer at 600 nm, while viable cells were determined using methylene violet staining and counted light microscopy (400 × magnification) using a Neubauer-type haemocytometer.

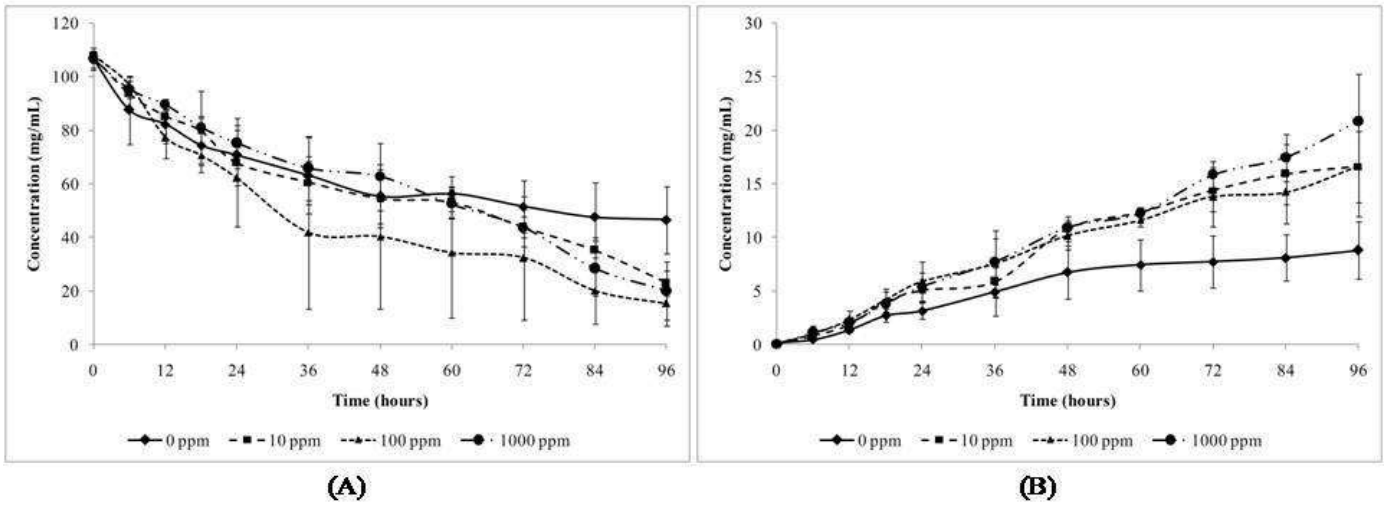

Figure 2: (A) glucose consumption and (B) ethanol production of baker's yeast cell grown in media without and with magnesium supplementation. Legends on the figure indicate the concentration of Mg^{2+}. Data presented are means of three replicates.

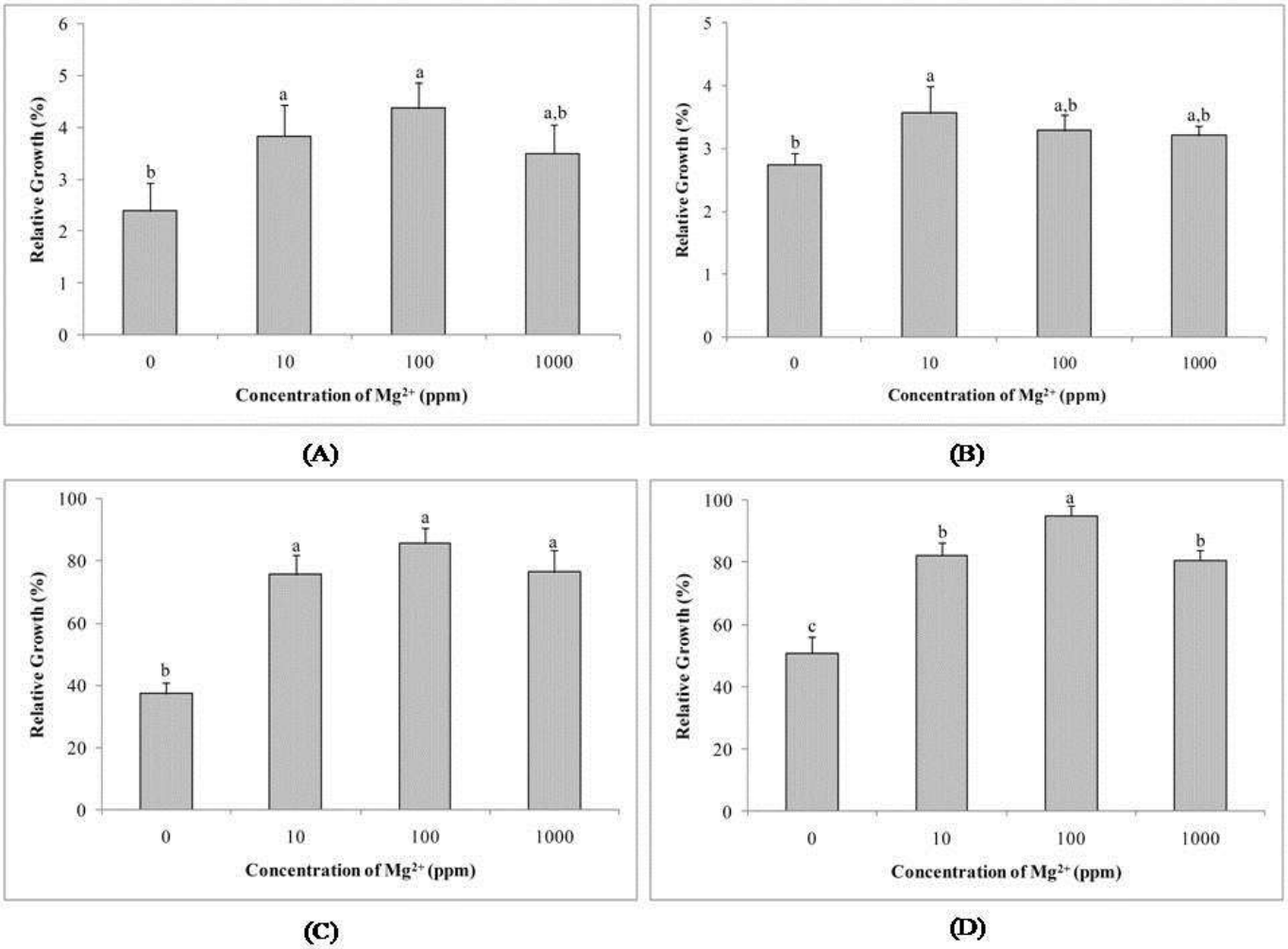

Figure 3: The effects of magnesium supplementation on baker's yeast cells grown in media with and without stress conditions. (A) Ethanol stress (B) weak acids stress, (C) hyperosmotic stress, (D) oxidative stress. Data presented are means of triplicate experiments of relative cell growths of cells grown in the presence of the stress factors compared to cells grown without stress factors. Error bars represent the standard deviation of the three independent experiments.

Table 1
Kinetic parameters of yeasts grown on YNB media with and without magnesium supplementation

Concentration of Mg^{2+} (ppm)	μ_{max}(/hours)	Q_s (mg/mL.hours)	Q_p (mg/mL.hours)	$Y_{p/s}$ (mg/mg)
0	0.070 ± 0.010^{b}	0.528 ± 0.082^{b}	0.095 ± 0.031^{b}	0.140 ± 0.010^{b}
10	0.179 ± 0.030^{a}	0.760 ± 0.062^{ab}	0.187 ± 0.034^{ab}	0.195 ± 0.027^{ab}
100	0.182 ± 0.028^{a}	0.885 ± 0.197^{a}	0.172 ± 0.046^{ab}	0.209 ± 0.025^{ab}
1000	0.181 ± 0.009^{a}	0.829 ± 0.050^{a}	0.215 ± 0.030^{a}	0.239 ± 0.035^{a}

Note: μ_{max}= maximum growth rate, Q_s = glucose consumption rate, Q_p= ethanol and $Y_{p/s}$ = ethanol yield

Conclusion

Magnesium ions can enhance the fermentation performance of yeast cells, as indicated by better specific growth rates, substrate utilization, ethanol productivity and ethanol yields. Magnesium also showed a protective effect against all stress factors tested in this study including ethanol, weak acid, hyperosmotic and oxidative stress.

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