The Influence of Treatment of *Saccharomyces cerevisiae* Inoculum with a Magnetic Field on Subsequent Grape Must Fermentation

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168-hour old *Saccharomyces cerevisiae* wine yeast cells on Petri dishes were exposed to a homogenous static magnetic field of 140 mT for periods of 24, 48 or 72 hours and then used as inoculum for the alcoholic fermentation of Malvasia grape must. The exposure to the magnetic field improved the fermentation process kinetics. Biomass and ethanol yields of fermentations inoculated with treated inoculum were higher than those in the control fermentation, which was inoculated with an untreated inoculum. Treatment of the inoculum with the magnetic field also led to faster consumption of glucose. Higher levels of ethanol, acetaldehyde, 1-propanol, 2-butanol, isoamil alcohol and lactic acid were detected. Faster consumption of tartaric acid was indicated, while no effect was identified in malic acid consumption.

Key words:
Saccharomyces cereviseae, inoculum magnetic field, wine fermentation

Introduction

From ancient times to the present day, in some wine regions, the influence of moon magnetism in grape must alcohol fermentation has been postulated possibly as a myth. The influence of Earth´s magnetic field on wine fermentation by *Saccharomyces cerevisiae* was observed in Roman times. It was found that variations in the magnetic field strength in different locations of the wine cellars influenced the alcoholic fermentation of the grape must.[1]

One of the first studies of the influence of magnetic fields on the growth of yeast cells during wine fermentation was that of Kimball, published already in 1937.[2] A suspension of wine yeast was exposed to a heterogeneous static magnetic field of 0.04 T for different times and the subsequent sprouting of the yeast cells was measured. Exposure for 10 to 17 minutes had no effect; while exposure for 20, 25, 30, 60, and 150 minutes inhibited sprouting. Yeast budding was only affected by heterogeneous fields; homogeneous fields produced no effect.[2]

Recently, there has been a resurgence of interest in the application of magnetic fields to yeasts, with various researchers applying magnetic fields stronger than that of Earth, which varies from 0.025 to 0.065 mT, depending on the location.[3–6] Beyond field strength of the magnetic field, it is of high relevance whether the field is homogeneous or heterogeneous, whether the field is static or alternating, and the process temperature.[4–6]

The results of the influence of magnetic fields on yeast growth and metabolism are contradictory. Some studies have not shown any effect. For example, there was no statistical difference between the growth of *S. cerevisiae* when cultured within the 1.5 T magnetic field of a clinical magnetic resonance imager, and when it was cultured outside of this magnetic field.[7] Likewise, growth of *S. cerevisiae* WS8105-1C was not affected by exposure to a static magnetic field of 50 Hz frequency, varying between 0.35 and 2.45 mT.[8,9] However, various studies have demonstrated effects. Exposure of a culture of *S. cerevisiae* to a magnetic field of 110 to 220 mT led to faster growth and higher respiration rates;[9,10] a culture of *S. cerevisiae* exposed to a 3 mT homogeneous magnetic field had a more porous membrane, absorbing 50 % more copper Cu^{2+} ions than non-exposed control cells;[11] growth of *S. cerevisiae* was reduced by exposure to an alternating 10 mT field at 50 Hz, and the surviving cells were more resistant to the ethanol production;[12] magnetic field exposed cells of *S. cerevisiae* immobilized on magnetic particles also showed higher ethanol production;[13] finally, cultivation of *S. cerevisiae* ATCC 7754 in a static magnetic field of 25 mT during 16 h led to a 20 % higher biomass

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concentration and a 39 % higher glutathione production compared to untreated cells.[14]

Unfortunately, these contradictory results make it impossible to state clearly what effect magnetic fields have on yeast growth. It has been suggested that the magnetic field influences cell membrane permeability, active transport through the cell membrane and protein synthesis.[12] It has also been suggested that magnetic fields can cause some essential molecules in the cell to move from their normal location, interrupting normal cell metabolism. The suggestion that the magnetic field influences the rate of chemical reactions or protoplasmic streaming is less probable.[2,8]

The main purpose of the present research was to investigate the influence of the static magnetic field on wine yeast cells metabolism and in subsequent grape must alcohol fermentation.

Material and methods

Microorganism

Saccharomyces cerevisiae (Daystar Ferment AG, CH – 6300 ZUG) was cultivated on Worth agar Petri dishes containing (in g L^{-1}): glucose 14.5, mineral salts $(NH_4)_2SO_4$ 4.06, $(NH_4)_2HPO_4$ 1.30, KCl 0.14, $MgSO_4 \cdot 7H_2O$ 0.30, $CaCl_2$ 0.55 and yeast extract 0.92.

Magnetic field

Petri dishes with 72 h culture of *Saccharomyces cerevisiae* yeast cells exposed to homogeneous static magnetic field of 140 mT, at 18 °C, were used in all the experiments. The magnetic fields were generated by a coil powered by a transformer. The coils were separated 1 cm and produced a homogeneous field in the vertical direction in the central area near the axis of the coils. Yeasts were located in the region within the coils where fields are homogeneous (Fig. 1).

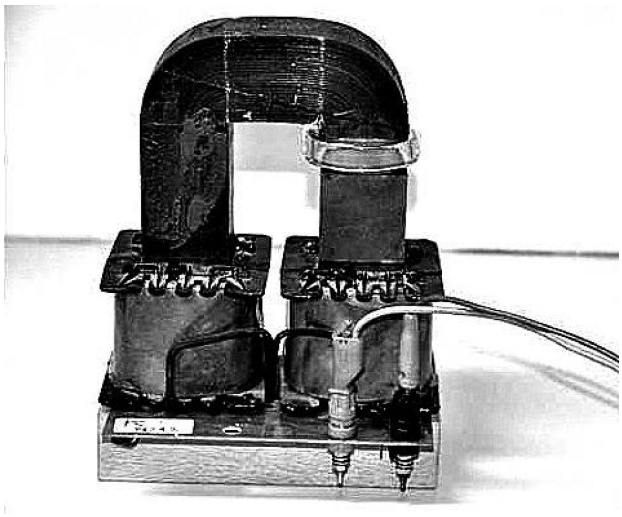

Fig. 1 – *Petri dish with 72 h culture of Saccharomyces cerevisiae yeast cells exposed to homogeneous static magnetic field of 140 mT, at 18 °C. Coil distance 10 mm*

Inoculum

As inoculum, a yeast cell suspension in concentration $2 \cdot 10^7$ cells mL^{-1}, previously for 24, 48 and 72 hours exposed to the static magnetic field of 140 mT, was used in all the experiments. Inoculum cells used in control experiments were grown for 24, 48 and 72 h, under identical conditions – but without the magnetic field.

Substrate

Malvasia grape juice from the Vipava wine-growing region was used as a fermentation media in all experiments. The musts, fermented on laboratory scale were not sulphurized before fermentation.

Fermentor

10 L stirred tank reactor of standard configuration was equipped with reflux cooler column, Mettler Toledo pH electrode (HA-405-DPA-SC-S8) and redox electrode (Pt4805-DPA-SC-S8), temperature control unit and agitation control (Bioengineering AG, Switzerland) was used. For *on-line* process control SHIVA control software (BIA d.o.o., Slovenia) was used. The fermentor's head space was aerated with N_2 to prevent oxidation of the fermenting grape must.

1. Fermentation

2. 10 L of grape must was inoculated at T = 18 °C and N = 100 rpm, with 20 mL yeast cell suspension previously exposed to homogeneous static magnetic field of 140 mT.

3. Analytical methods

Organic acids, reducing sugars, and alcohol in the wine and grape must were analyzed by HPLC. Standard validation methods proposed by BIO-RAD (1997) were applied. Samples were filtered through a 0.45 μm membrane and analyzed using 300 mm × 7.8 mm Aminex HPX-87H organic acid analysis cationic exchange column. Elution was performed at 65 °C. The mobile phase was 0.005 mol L^{-1} H_2SO_4 in bi-distilled water. The pump was operating at a flow rate of 0.5 mL min^{-1} ($0.008 \cdot 10^{-3}$ L s^{-1}). The injection volume was 20 μL. The eluting compounds were monitored at 210 nm by a fixed ultraviolet (UV-VIS) wavelength detector. This detector was connected in series with a refractive index (RI) detector. Tartaric and malic acids were detected by UV; citric, succinic acids, glucose, fructose,

glycerol and ethanol were detected by RI detector. The peaks were quantified using external standard calibration. The components were identified by a comparison of their retention times with those of the standards. Quantification was performed using external standards prepared from pure compounds.[15]

Acetaldehyde, iso-amyl alcohol, 1-propanol and 2-butanol analysis were performed by gas chromatography. Hewlett Packard 6890 gas chromatograph was used in all the experiments. Temperature of injector was 180 °C, and temperature of detector was 300 °C. The components were identified by comparison of their retention times with those of the standards. Quantification was performed using internal standard.

Biomass was determined gravimetrically after 5 minute centrifugation of 20 mL of fermentation broth at 4000 rpm and 24 h drying at 105 ºC.

TEM electron microscopy

Cell structure was examined using transmission electron microscope (TEM) Philips CM 100. Yeast cells were prepared for ultrastructural analysis by conventional method of fixation in a mixture of 1.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 mol L^{-1} phosphate buffer, postfixation in 1 % osmium tetroxide, dehydration in graded series of ethanols, and embedding in Spurr. Ultrathin sections were stained in uranyl acetate and lead citrate.

Statistics

All the experiments were performed in triplicate. The statistical significance was evaluated with the student t-test and significant results were considered at $P < 0.05$ unless stated otherwise (Tables 1, 2).

Results

On-line redox potential measurement was used as a monitor of yeast cell metabolic activity. It differed between fermentations inoculated with the treated and control cells. In all the experiments, the measurements started at 400 mV. For the fermentation done with the control inoculum, the aerobic phase lasted 24 h, with the potential reaching 430 mV. With the 24-h treated inoculum, the aerobic phase lasted 18 h, with the potential reaching 420 mV. The aerobic phase was very short with the other treated inocula: 15 minutes with 24 h treated inoculums, 10 minutes with the 48 h, and 6 minutes with the 72 h treated inoculum. With an increase in the magnetic field treatment, the final redox potential was lower and the time at which it was reached was shorter: The fermentations done with the control reached –130 mV at 108 h, 24 hour treated inocula –230 mV at 96 h, 48 h –300 mV at 72 h and at 72 hour treated inocula –395 mV at 36 h respectively (Fig. 2).

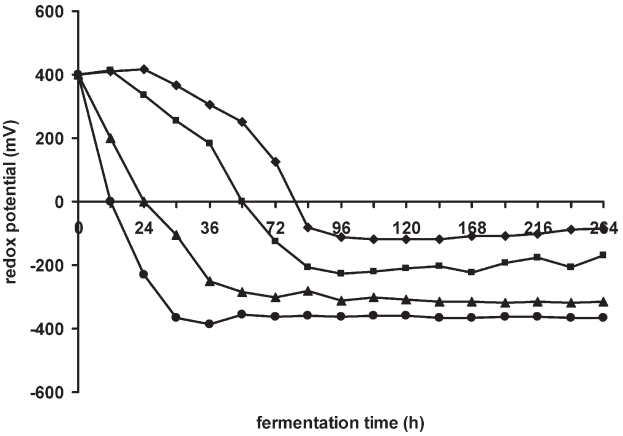

Fig. 2 – *Fermentation on-line redox potential time courses ♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h*

The biomass determinations showed similar results (Fig. 3). Biomass levels measured at 96 h, when all cultures had reached the stationary phase, increased with increasing treatment time of the inoculums to 3.78 g L^{-1} (24 h), 4.22 g L^{-1} (48 h) and 4.34 g L^{-1} (72 hour treated cells) compared to the control 3.39 g L^{-1} respectively (Table 1).

The rate of consumption of glucose increased with increasing pretreatment of the inoculum with the magnetic field (Fig. 4a). Glucose was essential-

Table 1 – *Final data on consumption of glucose and fructose, production of biomass, ethanol, glycerol and acetaldehyde in fermentation with exposed yeast inoculum cells and control*

	Biomass (g/l)	Glucose (g/l)	Fructose (g/l)	Ethanol (g/l)	Glycerol (g/l)	Acetaldehyde (g/l)
control	3,39±0,04	6,35±0,32	30,66±0,34	73,44±1,81	8,34±0,08	0,011±0,0002
magnetic field 1day	3,78±0,06	1,99±0,25	19,23±1,10	83,27±0,75	8,55±0,12	0,013±0,0004
magnetic field 2days	4,22±0,07	1,35±0,26	12,65±0,97	85,34±1,34	9,23±0,15	0,015±0,0002
magnetic field 3days	4,33±0,05	1,14±0,32	7,82±0,43	87,96±1,67	9,65±0,09	0,015±0,0001

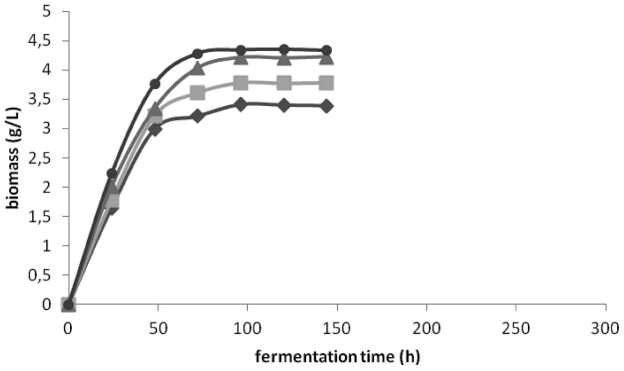

Fig. 3 – *Production of yeast biomass using various extents of inocula exposures*
♦ *control ; exposure :* ■ *24 h ;* ▲ *48 h ;* ● *72 h*

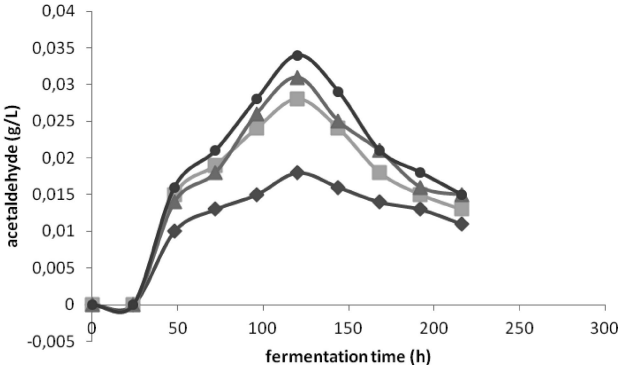

Fig. 5 – *Accumulation of acetaldehyde in fermentation time course*
♦ *control ; exposure :* ■ *24 h ;* ▲ *48 h ;* ● *72 h*

ly exhausted at 275 h in the culture inoculated with the control inoculum and at 235 h in the culture inoculated with the 72 hour treated inoculum. On the other hand, fructose consumption profiles were very similar for all fermentations, irrespective of the inoculum used (Fig. 4b).

In all cultivations, the acetaldehyde peaked at 125 h. Acetaldehyde production increased with increasing treatment of the inoculum with the magnetic field. For the fermentation carried out with the control inoculum, the peak acetaldehyde concentration was 0.011 g L^{-1}, while that with the 72 hour treated inoculum gave a peak of 0.015 g L^{-1} (Fig. 5).

Ethanol production was lower for the fermentation inoculated with the control inoculum. However, the final ethanol concentration did not vary significantly for the fermentations with the 24, 48 and 72 hour inocula (Fig. 6a). The glycerol production profiles for the fermentations with the treated inocula were not very different from those with the control inoculum (Fig. 6b). The production of iso-amyl alcohol, 1-propanol and 2-butanol increased with increasing length of the magnetic treatment of the inoculum (Figs. 7a, b, c).

In the fermentation with the control inoculum, tartaric acid levels were slightly lower than in the

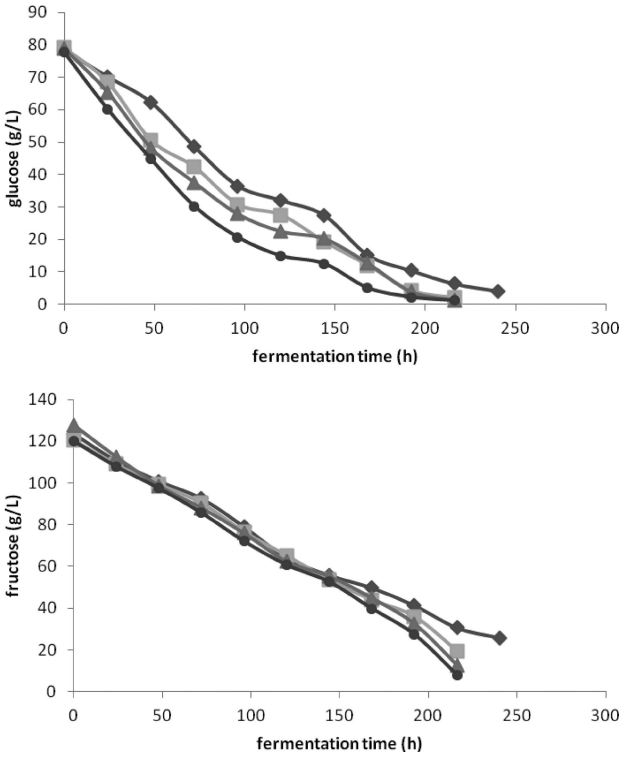

Fig. 4a,b – *Consumption of glucose and fructose in fermentation process*
a. glucose consumption, b. fructose consumption.
♦ *control ; exposure :* ■ *24 h ;* ▲ *48 h ;* ● *72 h*

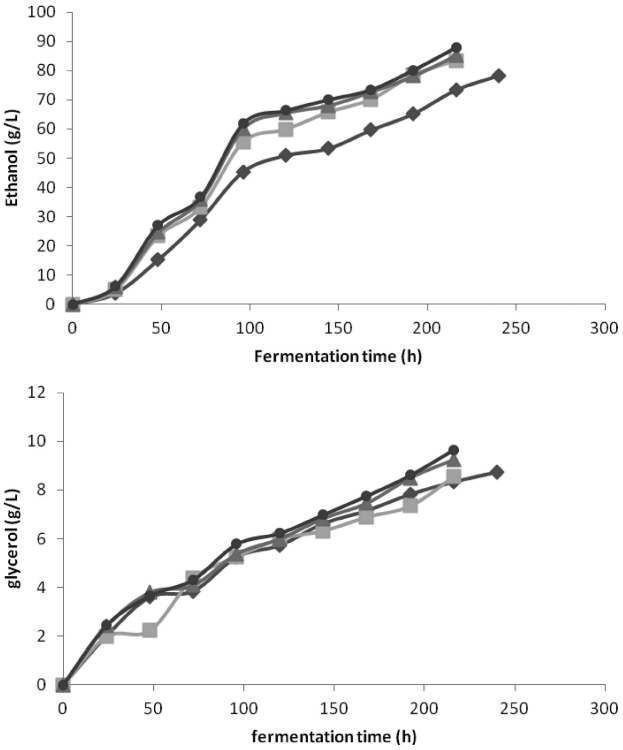

Fig. 6a,b – *Production of ethanol and glycerol in fermentation process*
a. ethanol, b. glycerol
♦ *control ; exposure :* ■ *24 h ;* ▲ *48 h ;* ● *72 h*

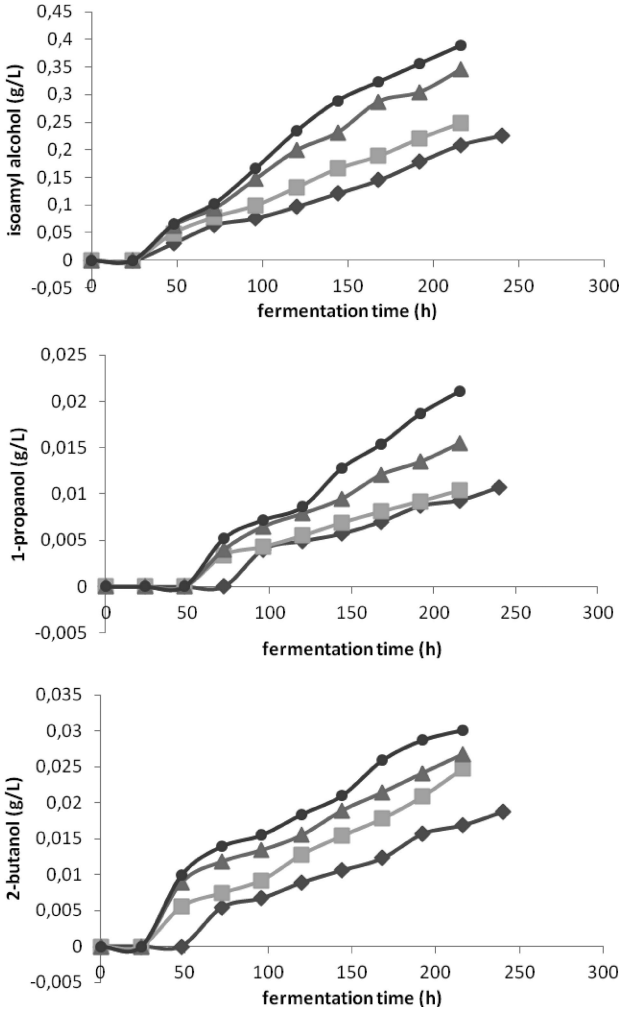

Fig. 7a,b,c – *Iso-amyl alcohol, 1-propanol and 2-butanol accumulation*
a. accumulation of iso-amyl alcohol, b. accumulation of 1-propanol, c. accumulation of 2-butanol;
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

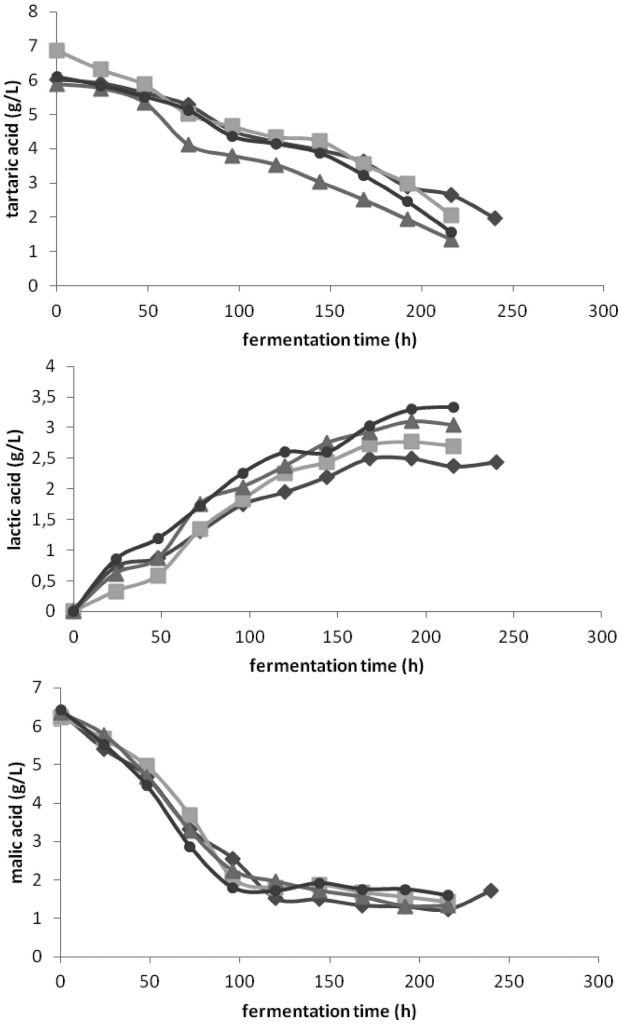

Fig. 8a,b,c – *Tartaric and malic acid consumption and accumulation of lactic acid*
a. Tartaric acid time course, b. malic acid time course, c. accumulation of lactic acid;
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

fermentations with treated inocula (Fig. 8a). In the case of malic acid consumption, there were no significant differences among the various experiments (Fig. 8b). Final lactic acid concentration increased with increasing magnetic treatment of the inoculum: with the control inoculum the final value was 2.37 g L^{-1}, compared to 3.34 g L^{-1} obtained in the fermentation with the 72 hour treated cells (Fig. 8c) (Table 2).

The TEM electron micrographs show that the cell shape and the ultrastructure of electro-stimulated yeast cells did not change compared to the control cells. Ultrastructural analysis showed that the fibrillar cell wall is composed of two layers, the

Table 2 – *Final data on consumption of malic and tartaric acid and production of lactic acid, 1- propanol, 2-butanol and isoamyl alcohol in fermentation with exposed yeast inoculum cells and control*

	Tartaric (g/l)	Malic (g/l)	Lactic (g/l)	1-propanol (g/l)	2-butanol (g/l)	Isoamyl a. (g/l)
control	2,66±0,11	1,24±0,005	2,37±0,006	0,0093±0,0004	0,0169±0,0011	0,209 ±0,001
magnetic field 1day	2,05±0,08	1,43±0,007	2,7 ±0,004	0,0104±0,0010	0,0248±0,0009	0,0249±0,002
magnetic field 2days	1,34±0,06	1,33±0,006	3,04±0,003	0,0155±0,0005	0,0267±0,0006	0,345 ±0,007
magnetic field 3days	1,56±0,09	1,6 ±0,003	3,34±0,008	0,0211±0,0006	0,0301±0,0008	0,389 ±0,005

Table 3 – *Influence of various types and strengths of magnetic field on S. cerevisiae cells*

Type	Strength	Effect	Reference
Homogeneous – static	3 mT	more porous membranes	11
Homogeneous – alternating	10 mT	resistance to the ethanol production	12
Homogeneous – static	25 mT	higher biomass concentration	14
Heterogeneous – static	40 mT	subsequent sprouting	2
Homogeneous – static	110–220 mT	faster growth and higher respiration rates	9–10
Homogeneous – static	140 mT	faster growth and kinetics	Present paper

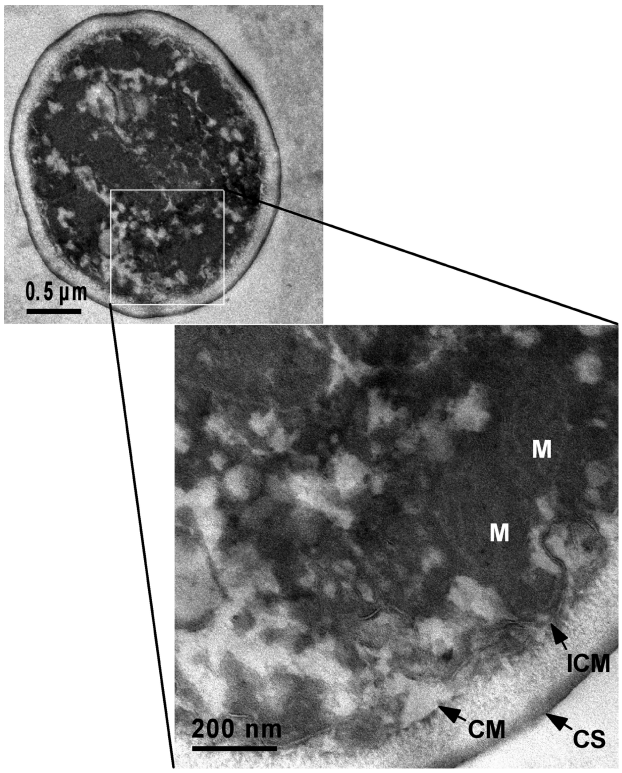

Fig. 9a,b – *Magnetic field exposed S. cerevisiae cell on the eighth day of wine fermentation. Mitochondria (M), cell membrane (CM) with numerous invaginations (ICM and multiple layer cell wall (CS) and electron lucent cytoplasma.*

periplasm is electron lucent and the cell membrane forms invaginations. The nucleus is surrounded by cellular inclusions, mostly lipid droplets and vacuoles are present (Figs. 9 a, b).

Conclusion

In the present research, the stimulating influence of homogeneous static magnetic field 10 mT on wine yeast biomass and cell metabolic activity in alcohol fermentation was found. According to the results, potentially favorable changes resulting in cellular metabolism and fermentation process kinetics have been observed. Summarizing the results and observations from the available publications [2,9–14] (Table 3) with the results and findings of the present research, the 168-hour *Saccharomyces cerevisiae* cell exposure does not cause noticeable changes in the cell structure itself, but it promotes faster growth and cell metabolism and therefore faster process kinetics. Although no significant changes in the cell structure were found, magnetic exposure also influences cell membrane permeability and motility; therefore the synergistic effect of all these factors has to be taken into the final account.

With an increase in the magnetic field, the length of the exposure resulted with lower fermentation process redox state, which resulted in a more stable and oxygen-resistant wine in a much shorter fermentation time. In the exposed samples, the highest biomass 4.33 g L^{-1} (28 % increase) was obtained in fermentation at 72 hour magnetic exposure, while at 48 h exposure 4.22 g L^{-1} (24 %), and in 24 h 3.78 g L^{-1} (11 % increase) amount of biomass compared to 3.39 g L^{-1} in control. Faster process kinetics was also found in the glucose consumption, while in the fructose consumptions results were quite similar for all three exposures. Although very similar results were obtained in final acetaldehyde production, it was evident that magnetic exposure influences the intensity of its biosynthesis. In alcohol production stimulation effect was recognized at ethanol, 1-propanol, 2-butanol and isoamil accumulation. Contrarily, in glycerol biosynthesis no significant influences compared to the control process were indicated.

In organic acids consumption, the fastest kinetics was identified for tartaric acid consumption at 48 h exposure, while for malic acid consumption no significant difference was indentified. The extention of magnetic exposure also promotes more expressed lactic acid production. Thus, 3.34 g L^{-1} (41 % increase) was detected in fermentation with 72 h exposure, 3.04 g L^{-1} (20 %) at 48 h, and 2.72 g L^{-1} (8 % increase) in 24 h exposure of yeast inocula cells compared to 2.37 g L^{-1} in the control. Similar to the 24 h, 48 h and 72 h magnetic inoculum treatments were the results of fermentations undertaken at 22 ºC, 24 ºC, 26 ºC, respectively.[18]

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