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

M. Berlot[1],T. Rehar[1], D. Fefer[2] and M. Berovic*[1],

[1] *Faculty for Chemistry and Chemical Technology, Department of Chemical, Biochemical and Ecology Engineering, University of Ljubljana, Aškerčeva 5, SI-1115 Ljubljana, Slovenia*

[2] *Faculty of Electrical Engineering, Department of Measurement and Robotics, Tržaška 25, 1000 Ljubljana, Slovenia*

Abstract

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 and higher levels of acetaldehyde, 1-propanol, 2-butanol, isoamil alcohol and lactic acid, and to decreased, but there was no effect on consumption of tartaric acid and malic acid.

Keywords: Saccharomyces cereviseae; inoculum magnetic field; wine fermentation

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1.Introduction

From the ancient times up now in some wine regions in grape must alcohol fermentation, the influence of moon magnetism was postulated already as a myth. The influence of the Earth´s magnetic field on wine fermentation by *Saccharomyces cerevisiae* was already observed in Roman times. It was found that variations of 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 min had no effect; while exposure for 20, 25, 30, 60, and 150 min 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 the Earth, which varies from 0.025 to 0.065 mT, depending on the location.[3-6] Beyond field strength, other important parameters are the strength of the magnetic field, whether the field is homogenous 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 concentration and a 39% higher glutathione production compared to untreated cells.[14]

Unfortunately, these contradictory results make it impossible to state clearly just what is the effect of magnetic fields 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 are 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 find out the influence of the static magnetic field on wine yeast cells metabolism 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 gl^{-1}): glucose 14.5, mineral salts $(NH_4)_2SO_4$ 4.06, $(NH_4)_2HPO_4$ 1.30, KCl 0.14, $MgSO_4.7H_2O$ 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 homogenous static magnetic field of 140 mT, at 22 °C, were used in all of the experiments. The magnetic fields were generated by a coil powered by a transformer. The coils were separated 1 cm and produce 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

Inoculum

As inoculums yeast cells suspension in concentration 2×10^7 cells/ml previously for 24, 48 and 72 hours exposed to the static magnetic field of 140 mT was used in all experiments. Inoculum cells used in control experiments were grown for 24, 48 and 72 h, under identical conditions – but without the magnetic field.

Substrate

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

Fermentation

10 l of grape must was inoculated at T = 22 °C and N = 100 rpm, with 20 ml yeast cells suspension previously exposed to homogenous static magnetic field of 140 mT. Yeast cell multiplication in fermentation was measured after 24, 48 and 72 hours using hemocytometer. The experiments were done in triplicate and the averages of the three runs were calculated.

Analytical methods

Organic acids, reducing sugars, and alcohol in 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 ºC. The mobile phase was

0.005M H_2SO_4 in bi-distilled water. The pump was operating at a flow rate of 0.5 ml/min (0.008×10^{-3} l/s). 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]

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

Results

On-line redox potential measurements differed between fermentations inoculated with the treated and control cells. In all of the experiments redox potential measurements started from 400 mV. For the fermentation done with the control inoculum, the aerobic phase lasted 24 h, with the potential reaching 430 mV (Fig. 1). 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: 10 min with the 48-h treated inoculum and 6 min with the 72 hour 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, 24-h, 48-h and 72 hour treated inocula reached -130 mV at 108 h, -230 mV at 96 h, -300 mV at 72 h and -395 mV at 36 h, respectively (Fig. 2).

Fig.2

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 3.82 g/L (24-h), 4.20 g/L (48-h) and 4.48 g/L (72 hour hours treated cells) compared to the control: 3.30 g/L respectively.

Fig.3

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

Fig.4 a,b

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 undertaken

with the control inoculum the peak acetaldehyde concentration was 0.0175 gl^{-1}, while that undertaken with the 72 hour treated inoculum gave a peak of 0.038 gl^{-1} (Fig.4).

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 undertaken with the 24-h, 48-h and 72 hour inocula (Fig. 6a). The glycerol production profiles for the fermentations undertaken with the treated inocula were not very different from that undertaken with the control inoculum (Fig.6b). The production of iso-amyl alcohol, 1-propanol and 2-butanol and increased with increasing length of the magnetic treatment of the inoculum (Figs. 7a,b,c)

Fig.6 a,b

Fig.7 a, b, c

In the fermentation undertaken with the control inoculum tartaric acid levels were slightly lower than in the fermentations undertaken with the treated inocula (Fig. 8a). In the case of malic acid consumption, there were no significant diferences 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 2.4 gl^{-1}, compared to a value of 3.4 gl^{-1} obtained in the fermentation undertaken with the 72 hour treated cells (Fig 8c).

Fig.8 a, b, c

Discussion

In the present research, the stimulative influence of static magnetic field on wine yeast cell multiplication, growth of biomass and its metabolic activity in alcohol fermentation was found. Potentially favorable changes in intracellular processes have been observed including changes in enzymatic activities, growth and respiration rates, increased motility and membrane permeability, and in morphological and developmental effects.

Comparing redox potential measurements in fermentation using yeast with magnetic exposure to control experiments faster fermentation kinetic with more intensive microbial activity was indicated. With an increase in the magnetic field treatment the length of the aerobic phase has significantly shortened and the final redox potential was lower, that indicates more stable and oxygen resistant wine in much shorter time.

In exposed samples, for 36 % higher biomass was obtained in fermentation with 72 hour magnetic exposure. Faster kinetics was indicated also in glucose consumption while in fructose consumptions quite similar results were obtained in all of the three exposures, quite similar and closer to the control sample.

Although very similar results were in 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. In contrary in glycerol biosynthesis it is evident that this process is less dependent on the magnetic field exposure.

In consumption of organic acids in case of tartaric and malic acid consumption there were no significant differences indicated. While extending the magnetic exposure on yeast cells promote more expressed lactic acid production.

Concerning to our results we could conclude that this results actually indicate a stimulatory effect of a treatment of the inoculum with a homogenous static magnetic field of 140 mT on *Saccharomyces cerevisiae* metabolism in a subsequent wine fermentation. The answer on faster and more productive cell metabolism is in assumation that magnetic field increases yeast membrane fluidity and, therefore facilitate the transport of metabolites out of the cells. This stimulatory effect of pretreatment with a magnetic field on the growth of wine yeast is very similar to the influence of cultivation at higher temperature. The results for the fermentations undertaken with 24 h, 48 h and 72 h magnetic treatments of the inocula are similar to those of fermentations carried out at 22 ºC, 24 ºC, 26 ºC, respectively.[18]

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Figures

Fig. 1 Petri dish with 72 h culture of *Saccharomyces cerevisiae* yeast cells exposed to homogenous static magnetic field of 140 mT, at 22 °C. Coil distance 10 mm.

Fig. 2 Fermentation *on-line* redox potential time courses.

♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig. 3 P*roduction of yeast biomass using various extend of the inocula exposures.*

♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig. 4 a,b Consumption of glucose and fructose in fermentation process.

a. glucose consumption, **b.** fructose consumption.

♦ 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

Fig.6 a,b Production of ethanol and glycerol in fermentation process

a. ethanol , **b.** glycerol ♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig. 7 a,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. 8 a,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

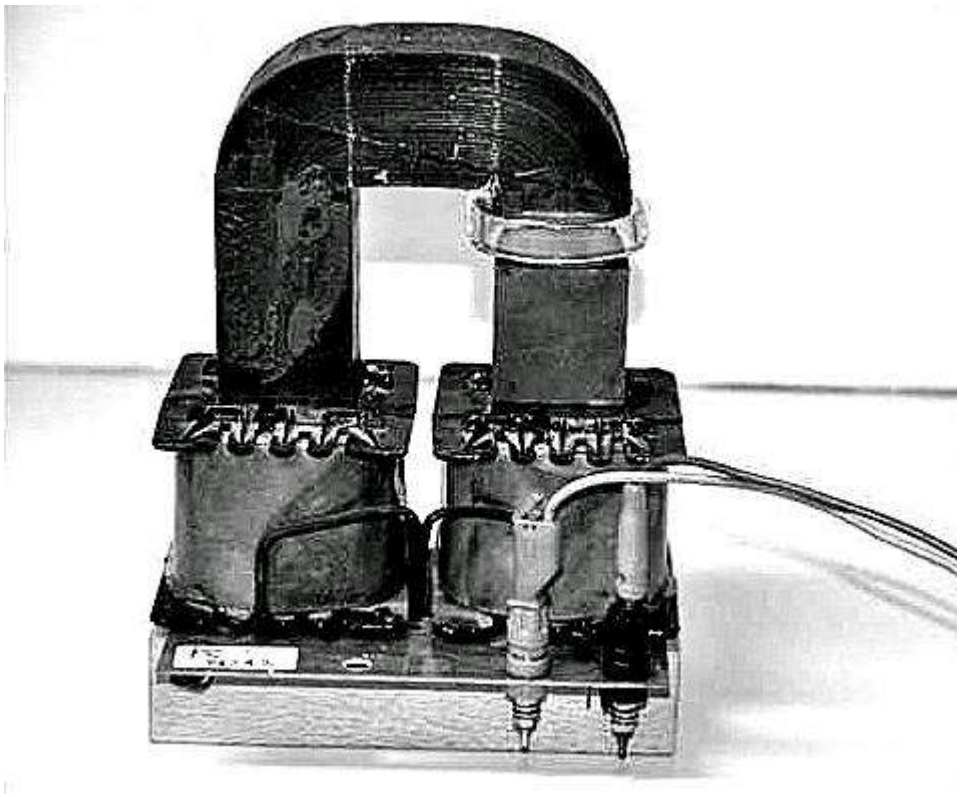

Fig.1

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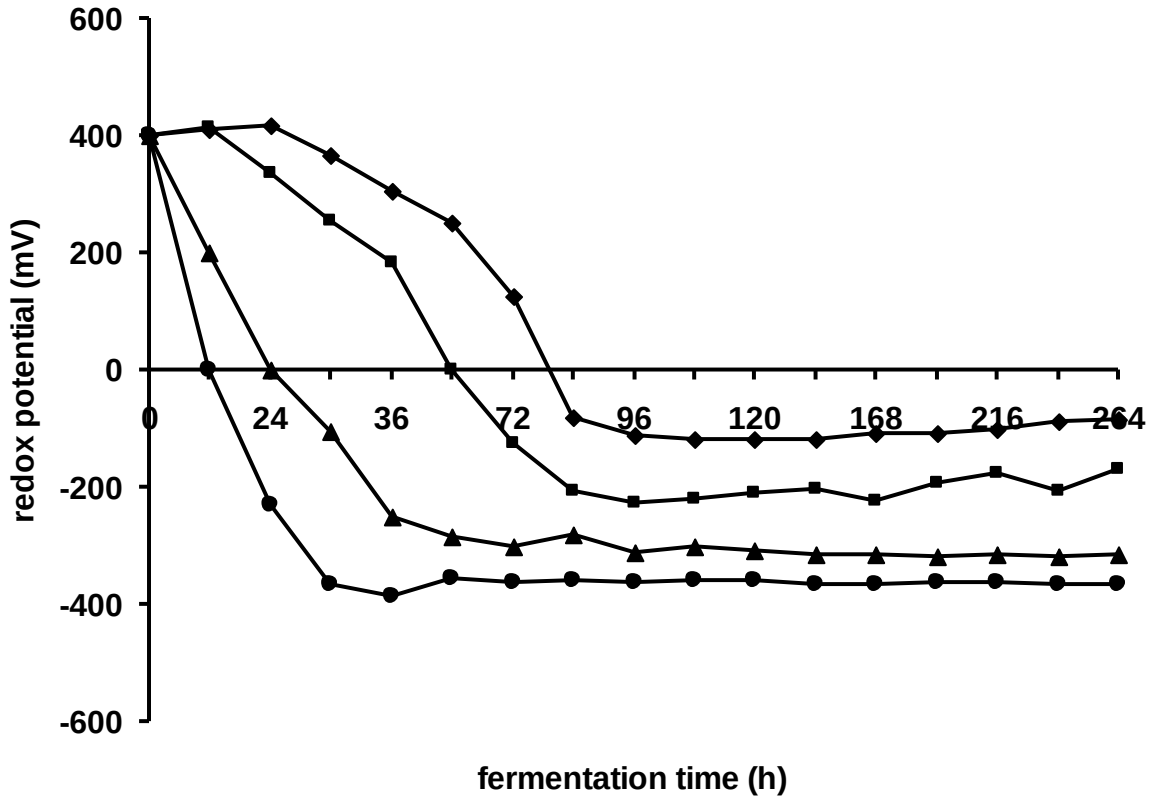

Fig. 2

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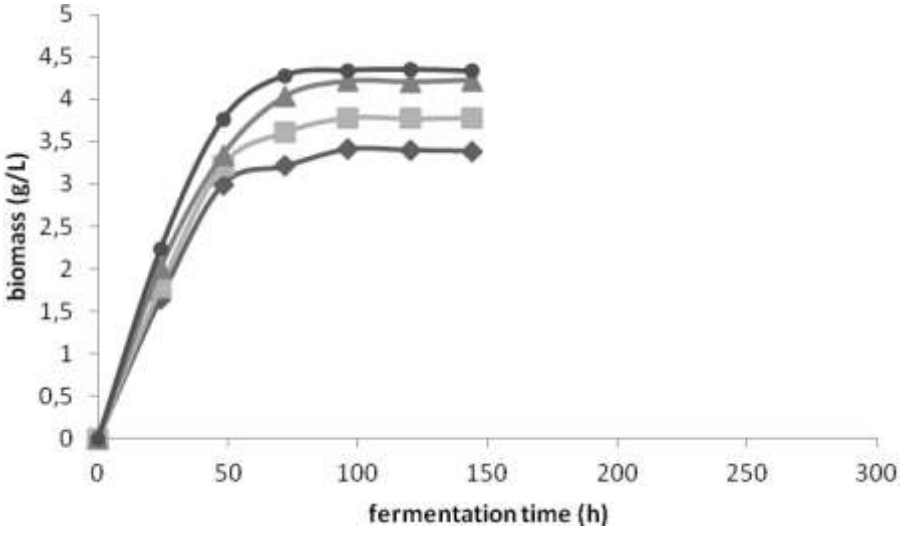

Fig. 3

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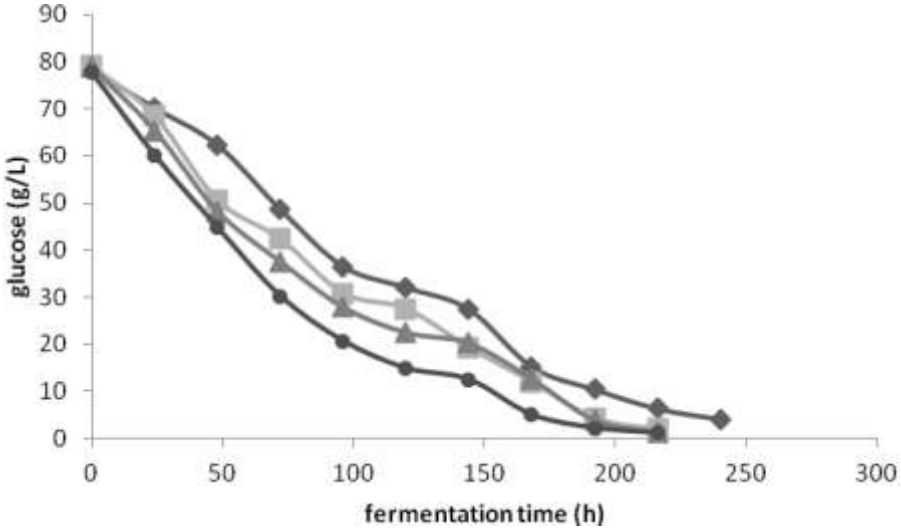

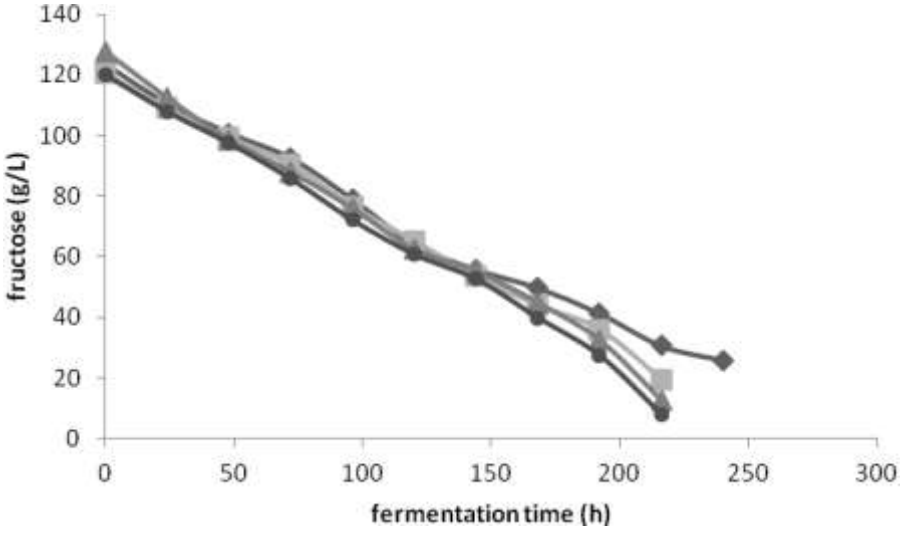

Fig. 4 a,b

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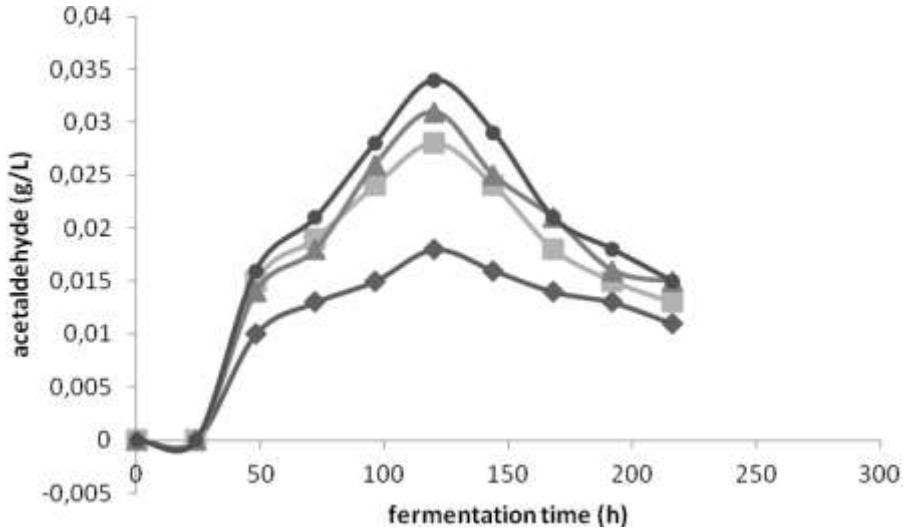

Fig. 5

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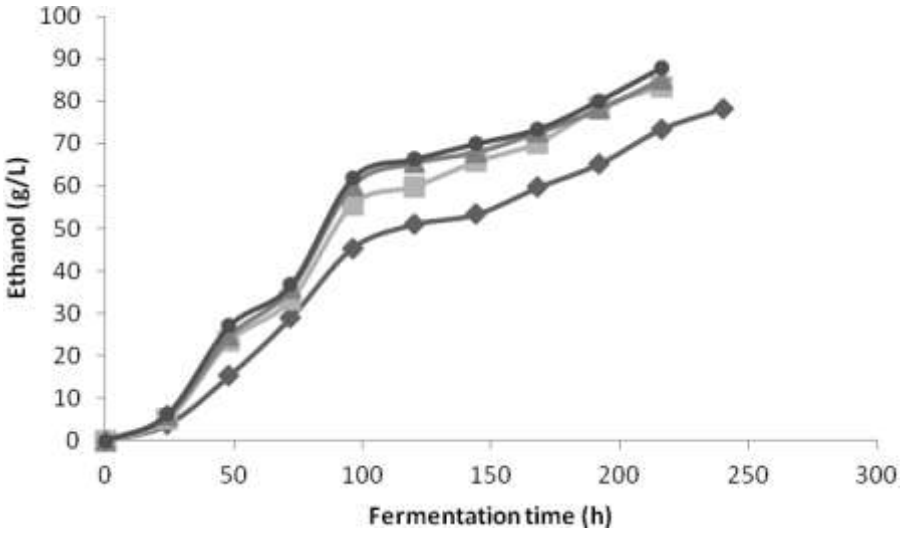

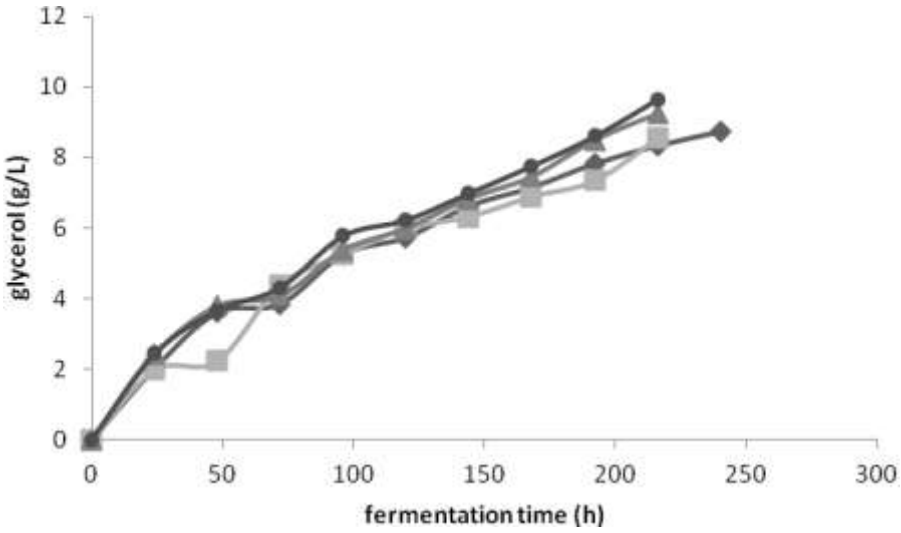

Fig. 6 a,b

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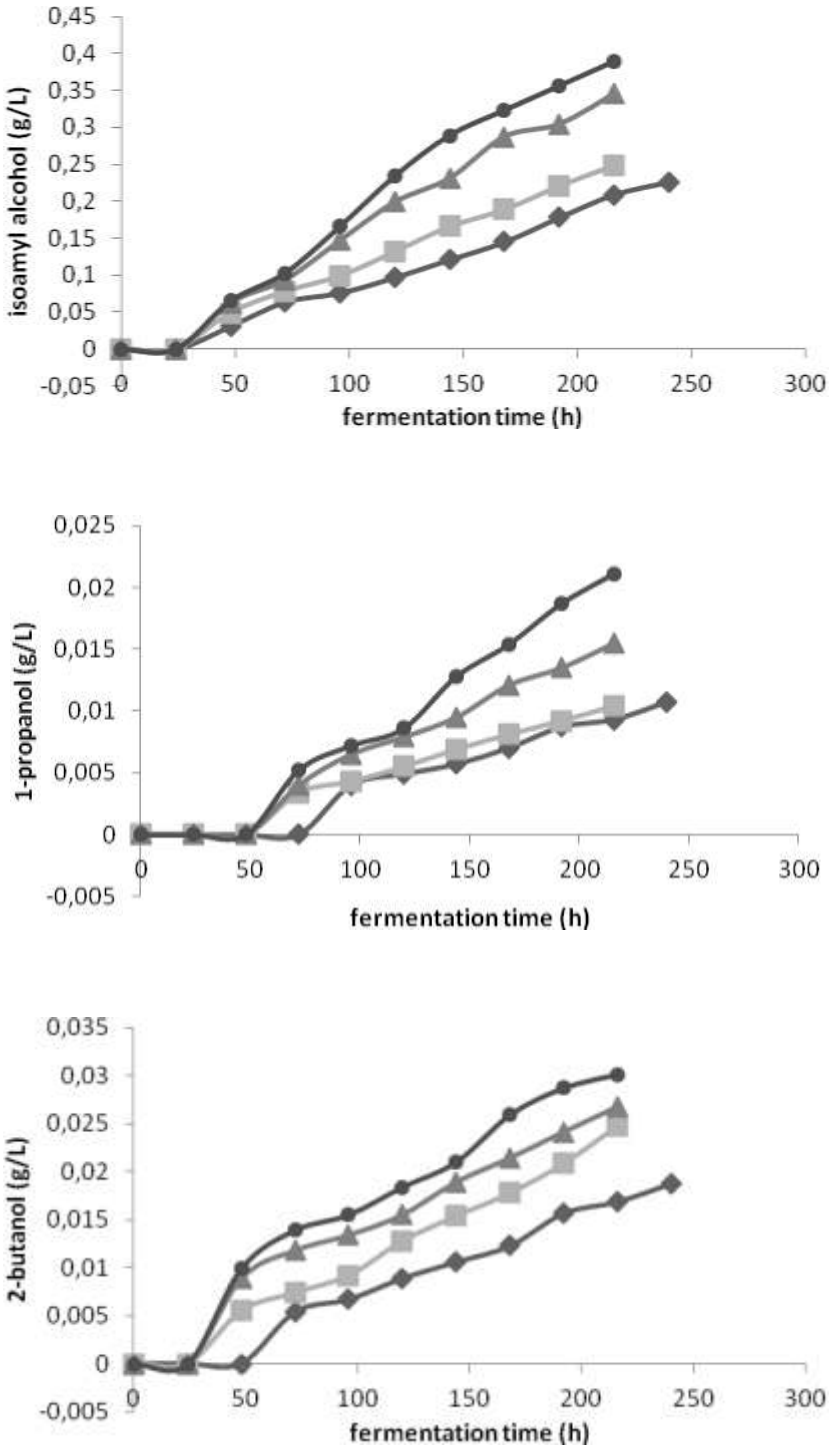

Fig. 7 a,b,c

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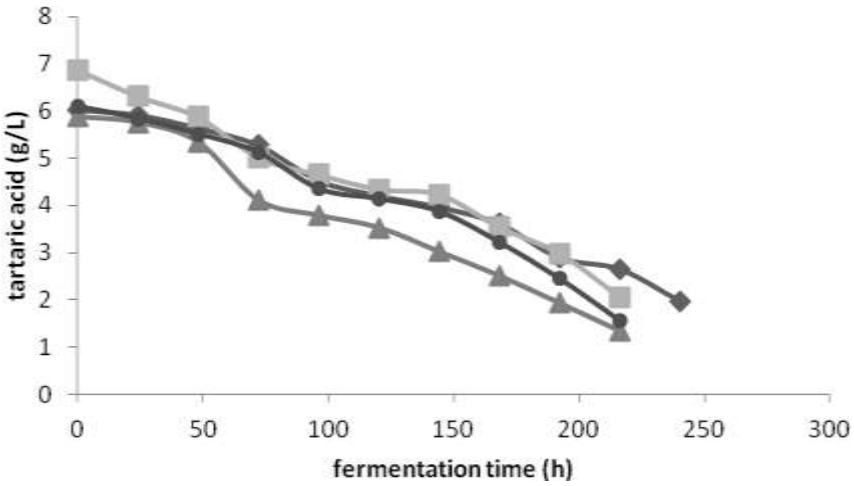
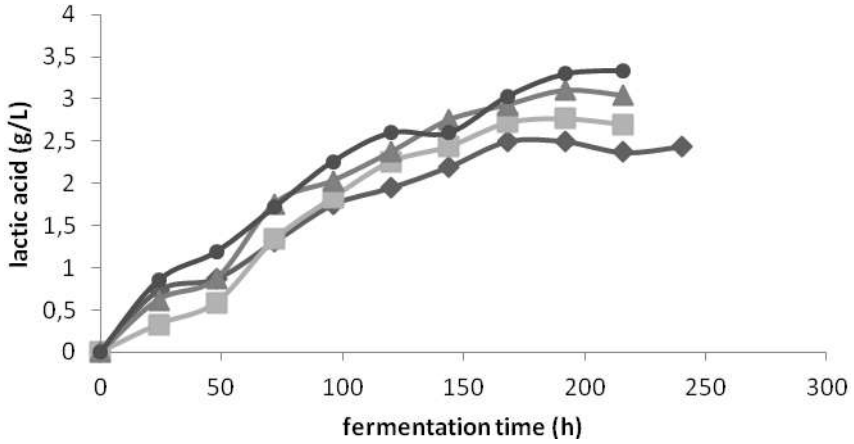

tartaric acid (g/L)
fermentation time (h)


lactic acid (g/L)
fermentation time (h)

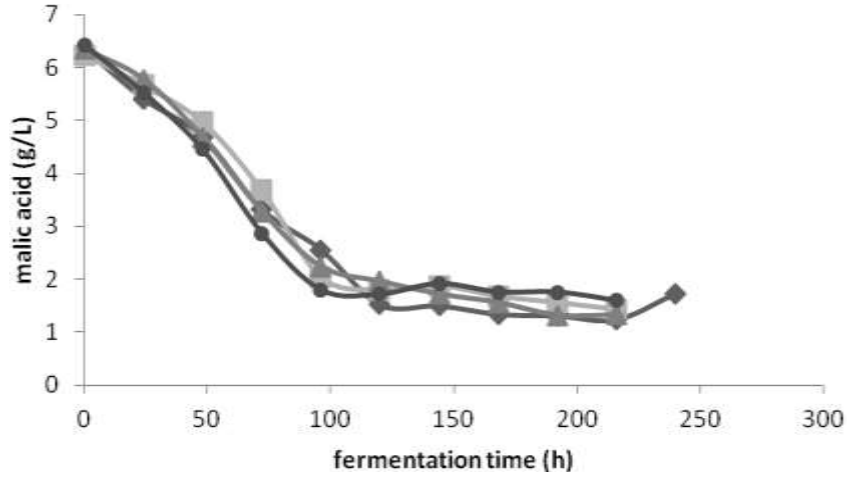

malic acid (g/L)
fermentation time (h)

Fig. 8 a,b,c