The Influence of Magnetic Field on *Saccharomyces cerevisiae* in Inoculum on Grape Must Fermentation

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Summary

7 days *Saccharomyces cerevisiae* wine yeast cells on Petri dishes were for 24, 48 and 72 hours exposed to homogenous static magnetic field of 140 mT and used in alcohol fermentation of Malvasia grape must. The results of analysis with high performance liquid chromatography (HPLC) indicated that the extend of the exposure promoted fermentation process kinetics. In exposed samples higher biomass and more intensive cell multiplication was detected. At 72 hour exposure fastest consumption of glucose and the higher acetaldehyde 1-propanol, 2-butanol and isoamil alcohol and ethanol accumulation were detected. Consumption of tartaric and malic acid is decreasing lowlier, lactic acid production is more expressed while in malic acid consumption there are no significant differences.

Keywords: *Saccharomyces cerevisiae*; alcohol fermentation; magnetic stimulation ; fermentation process kinetics; glycerol

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Introduction

Influence of earth magnetism field on *Saccharomyces cerevisiae* in alcohol fermentation on microbial physiology was already observed in Roman times. Earth magnetism, 0.03 - 0.07 mT, in different locations of the wine cellars influenced changes in alcohol fermentation of the grape must in wine (Funk *et.al*., 2009).

In past, various kind of research was focused in study of this phenomena (Fojt et al. 2009, Egami *et al.*2010). Related to this influence of magnetic field on cell membrane permeability and limitation of active transport in cell membrane, protein synthesis and gene expression were studied (Stašak *et al.* 2002).

The results of the influence of magnetic field in present research are often contradictorily. It is well important fact in this research the strength of the magnetic field, if homogenous or heterogeneous, static or oscillating magnetic field and what kind of process temperature was applied (Arnold *et al.* 2000, Otabe *et al*.2009, Egami *et al.* 2010).

One of the first studies of the influence of magnetic field on growth of the yeast cells in wine fermentation was published already by Kimball in 1937. Suspension of wine yeast was exposed to heterogenous static magnetic field 0.04 T. The results shown that heterogenous magnetic field inhibite the sprouting the yeast cells (Kimball 1937). Five minutes exposure causes an inhibition; 10, 15, and 17 minutes produce no effect; 20, 25, 30, 60, and 150 minutes are inhibiting. Any effect on the yeast buds is always associated with a heterogeneous field. Homogeneous fields produce no effect. The most probable explanation is that some essential molecules in the cell are moved from their location, thus interrupting the normal progress of anabolism. Less probable seems the assumption that the magnetic field influences the rate of chemical reactions or of protoplasmic streaming.

Malko *et al.*1994 used a clinical magnetic resonance imager to search for the possible effects of a 1.5 T magnetic fields on the growth of the yeast *Saccharomyces cerevisiae*. Yeast samples were grown in nutrient broth contained in constant-temperature boxes, both in and out of the magnetic field of the imager. No convincing statistical evidence for an effect of magnetic field on cell density was detected.

Motta, 2001, 2004 exposed yeast cells at 25° C to the magnetic field of strenght of 110 in 220 mT. Fastest growth of yeast biomass as well as higher respiration was detected at the cells exposed to the magnetic field of 220 mT (Motta, 2001, 2004).

Gorobets *et.al* (2006) studied the influence absorption of copper ions with *S. cerevisiae* at T = 28° C in homogenuous magnetic field of strenght 3 mT. Magnetic field was indicated to influence on porosity of membranes. Yeast cells exposed in the magnetic field absorbed for 50 % more copper ions than non exposed control cells from the tested solution.

Ruiz-Gomez *et.al* (2004) exposed *Saccharomyces cerevisiae* WS8105-1C cells from 24 to 72 hours to static magnetic field of 50 Hz frequency. Magnetic field was generated with a pair of Helmholtz coils (D = 40cm) with 0,35 in 2,45 mT. It was found that static and 50 Hz magnetic fields of 0,35 and 2,45 mT have no effect on the growth of *Saccharomyces cerevisiae*. Strašak *et.al* (2002), found that at temperature 24-26 °C cylindrical coil of 10 mT and a frequency of 50 Hz significantly reduces the growth of *Saccharomyces cerevisae* cells. Surviving cells the minority - old and too young cells – while the majority – the resistant cells multiplied on.

Liu *et.al* (2009) experimented in alcohol fermentation with immobilized cells of *Saccharomyces cerevisiae* on magnetic particles. Immobilized culture shown significant activity of ethanol production. This ability was influenced by concentration of reductive sugars as well as with a step of dilution rate. Santos *et.al* (2010), found that static magnetic field of 25,0 mT influences increasing of on wine yeast *Saccharomyces cerevisiae* ATCC 7754biomass and their ability of the glutathione production. 16 hour application of the magnetic field raised up the biomass concentration for a 19,6% and glutathione production for a 39 %.

Inconsistent results of other inactivation studies, however, make it impossible to clearly state the microbial activation or inactivation efficiency of magnetic field or to make any further predictions about its effects on microbial populations. The main purpose of present research was to find out the efficiency of the static magnetic field on wine yeast cells and it further use in alcohol fermentation of grape must to wine.

Material and methods

Microorganism

Saccharomyces cerevisiae yeast (Daystar Ferment AG, CH – 6300 ZUG) was cultivated on worth agar Petri dishes including glucose 14,5 g/l, mineral salts $(NH_4)_2SO_4$ 4,06 g/l, $(NH_4)_2HPO_4$ 1,30 g/l, KCl 0,14 g/l, $MgSO_4 \cdot 7\ H_2O$ 0,30 g/l $CaCl_2$ 0,55 g/l) and yeast extract 0,92 g/l.

Magnetic field

Petri dishes with *Saccharomyces cerevisiae* yeast was at T = 22 °C exposed to homogenous static magnetic field of 140 mT used in all of the experiments.

Inoculum

5 ml sterile 0.4% NaCl was added to for 24 hours *Saccharomyces cerevisiae* biomass on Petri dishes. In further they were exposed for 24, 48 and 72 hours to the static magnetic field of 140 mT.

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, at fermentation temperature were not sulphurized before the beginning of the fermentation.

Fermentor

10 l stirred tank reactor of standard configuration (Bioengineering AG, Switzerland) was used. It was equipped with reflux cooler column, Ingold pH and redox electrodes, temperature control unit and were stirred at 100 rpm. For *on-line* measurements, SHIVA control software (BIA d.o.o., Slovenia) was applied. The fermentors' head space was aerated with N_2 to prevent oxidation of the fermenting grape must.

Fermentation

10 l of grape must was at T = 22 °C inoculated with 20 ml of 24 hours yeast suspension in concentration $2 \cdot 10^7$ cells/ml suspension of the yeast cells previously exposed for 24, 48 and 72 hours to the static magnetic field of 140 mT was used in all experiments. Yeast cell multiplication in grape must alcohol fernentation was measured after 24, 48 and 72 hours using hemocytometer.

Analytical methods

Organic acids, reductive sugars and alcohol in wine and grape must were analysed by HPLC. Standard validation methods proposed by BIO-RAD (1997), were applied. Measurements of the concentrations of reductive sugar, ethanol, glycerol, concentrations of some organic acids and biomass concentration were *off-line* daily measured. Samples were filtered through a 0,45 μm membrane and analysed 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 ($0{,}008 \cdot 10^{-3}$ 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.

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

Results and discussion

Influence of static magnetic field on growth and metabolic activity of exposed yeast cells in in grape must alcohol fermentation was monitored over *on-line* redox potential measurements. Differences between *on-line* redox potential measurements

and the control samples were observed. Redox potential measurement at control and at 24 hour exposed cells fermentation indicates short aerobic phase. In control fermentation this phase lasted for 24 hours and it started from 400 mV and ended at at 430 mV. At 24 hours exposure this phase was 18 hours. In this period redox potential rised up to 420 mV. In fermentations with longer exposed cells for 48 and 72 hours the aerobic phases were evidently shorter. They proceeded in 10 and 6 minutes.

After this redox potential drops to negative values that indicates that yeast is turning to anaerobic ethanol production phase. Minimas in redox potential measurements were indicated - 130 mV at 108 hours in the control fermentation, - 230 mV at 96 hours (24 hours exposure), - 300 mV at 72 hours (48 hours exposure) and - 395 mV at 36 hours at cells with 72 h exposure in magnetic field (Fig. 1).

Fig. 1

Exposed yeast cells were in further used as inoculum in grape must fermentation. Cell multiplication was measured in fermentation was measured after 24, 48 and 72 hours. The average results from each three run were sumarized and presented in Table 1. The results showed that multiplication was stimulated cell multiplication in all theree cases. At 24 h exposure the increasement was for 28.5 %, at 48 h 27.4 while the most expressed for 30 % was at 72 h exposure.

Table 1

Measurement of wine yeast biomass showed that in all magnetic field treated inoculum cells biomass was also higher comparing to the control. The highest biomass was indicated in all cases after 96 hours of fermentation 4.48 g/l sample with 72 hour, 4.20 g/l at 48 hour and 3.42 g/l at 24 hours of magnetic field exposure (Fig.2).

Fig.2

Figs.3a,b presents consumption of glucose with the inoculum of yeast cells 72 hours exposed into static magnetic field compared with the consumption in control

experiment. The rate of glucose and fructose consumption in fermntation with yeast cells exposed in magnetic field is much faster. Consumption of glucose is again the fastest at 72 hours where at 235 hours is glucose mostly consumed, compared to control fermentation where at 275 hours glucose was completely consumed.

Figs.3a,b

In consumptions of fructose all of the three courses where magnetic field was applied were quite similar and quite closer to the control sample (Fig.3b).
Acetaldehyde production in all run reached its maximum at the same point after 125 hours of fermentation. The most expressed was 0.038 g/l at 72 hours of exposure while the lowest indication was 0.0175 g/l at the control sample (Fig.4).

Fig.4

HPLC analysis of ethanol acummulation also indicated the highest production 9.8 g/l at the cells with 72 hours magnetic field exposure (Fig.5).

Fig.5

The course of glycerol biosynthesis is in all of the samples very similar to those of the ethanol (Fig.6), but the kinetic of its generation shows that the synthesis of glycerol is on the influence of the magnetic field less dependent.

Fig.6

The highest concentrations of 1-propanol, 2-butanol, isoamil alcohol were also detected at the longest exposure of the yeast cells (Fig 7 a,b,c).

Figs. 7 a,b,c

From the fermentation courses of organic acids it is evident that tartaric acid is decreasing slowlier at exposed yeast cells (Fig.8a) while in malic acid consumption there are no significant diferences between all of the experiments (Fig.8b). Lactic

acid production 3.4 g/l is also more expressed at the longest exposure to magnetic field compared to 2.4 at the control experiments (Fig 8c).
Figs.8a,b,c

Conclusions

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 critical process factors affecting the inactivation of microbial populations by magnetic fields are not completely understood. Some factors believed to influence microbial inactivation include magnetic field intensity, electrical resistivity, and microbial growth stage.

In present research the simulative influence of static magnetic field on wine yeast cell multiplication and on growth of the biomass and its metabolic activity in alcohol fermentation was found.

Comparing redox potential measurements in fermentation with yeast with magnetic exposure to control experiments faster fermentation kinetics with more intensive microbial activities was indicated. In exposed samples higher biomass and more intensive cell multiplication was detected. At 72 hour exposure fastest consumption of glucose was indicated while fructose consumptions was in all of the three exposures quite similar and closer to the control sample.

Magnetic exposure for 72 hours stimulate also acetaldehyde 1-propanol, 2-butanol and isoamil alcohol production as well as also ethanol accumulation, while the kinetic of glycerol synthesis shows that this process is magnetic field less dependent.

Consumption of organic acids, tartaric and malic acid is decreasing slowlier with extend of yeast cells magnetic exposure while in malic acid consumption there are no significant diferences between all of the experiments. Lactic acid production is also more expressed with the extend of magnetic exposure.

Exposure to a magnetic field may stimulate or inhibit the growth and reproduction of microorganisms (Hoffman 1985). High intensity magnetic fields can affect membrane fluidity and other properties of cells (Frankel & Liburdy 1995).

However, the stimulation effects on *Saccharomyces cerevisiae* wine yeast biomass its metabolism and kinetics are very similar to those performed at higher temperature, where 24 hours inoculum cell exposure activity could be compare to

results obtained with higher fermentation temperatures. Results of 24 hours magnetic exposure to results of fermentation metabolism at 22 ºC, 48 hours to 24 ºC and 72 to T = 26 ºC (Berovic *et al* 2003).

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Figures

Fig. 1 Fermentation *on-line* redox potential courses.
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h
.

Fig. 2 Biomass production.
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig. 3 a,b **a.** glucose consumption, **b.** fructose consumption.
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig. 4 Accumulation of acetaldehyde in fermentation time course.
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig.5 a,b **a.** Production of ethanol , **b.** production of glycerol
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

Fig. 6 a,b,c **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. 7 a,b,c **a.** Tartaric acid time course, **b.** malic acid time course,
c. accumulation of lactic acid
♦ control ; exposure : ■ 24 h ; ▲ 48 h ; ● 72 h

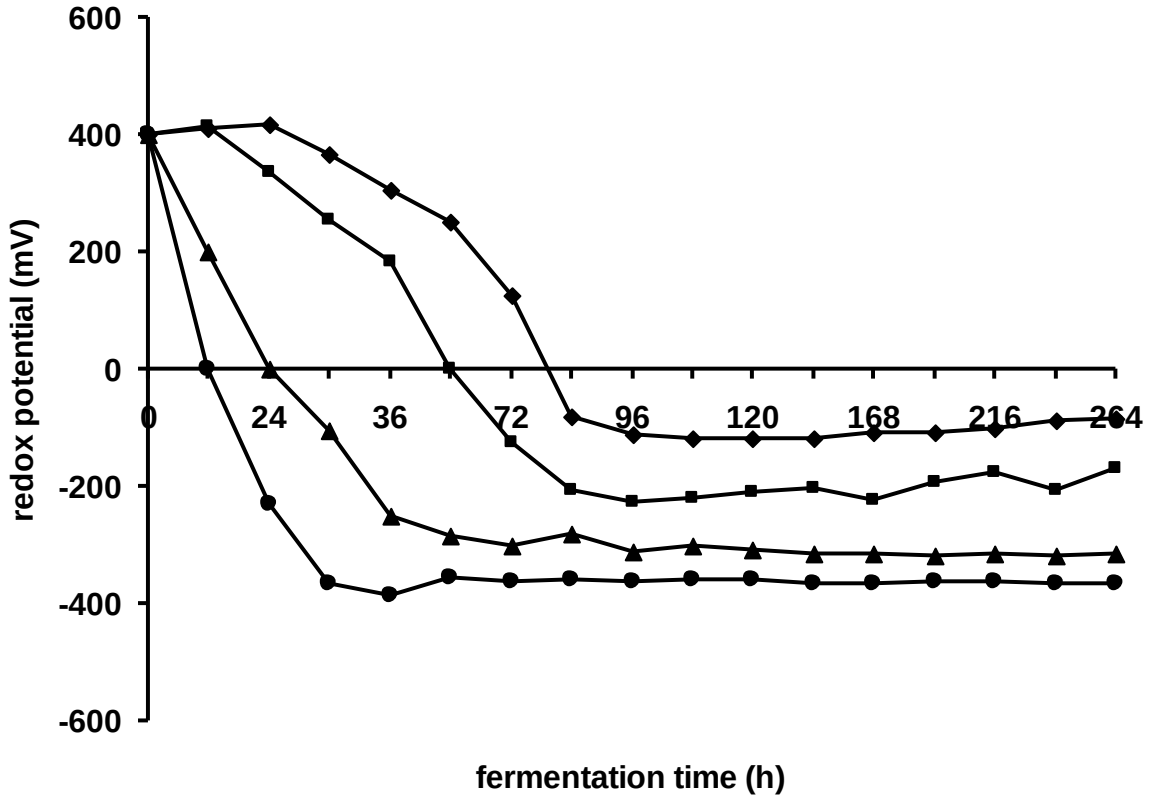

600
400
200
0
-200
-400
-600
redox potential (mV)
0
24
36
72
96
120
168
216
264
fermentation time (h)

Fig. 1

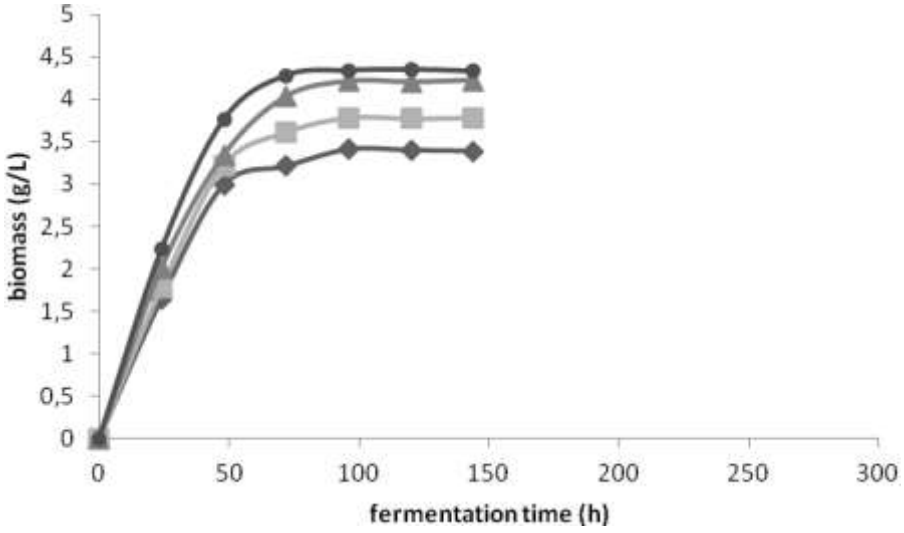
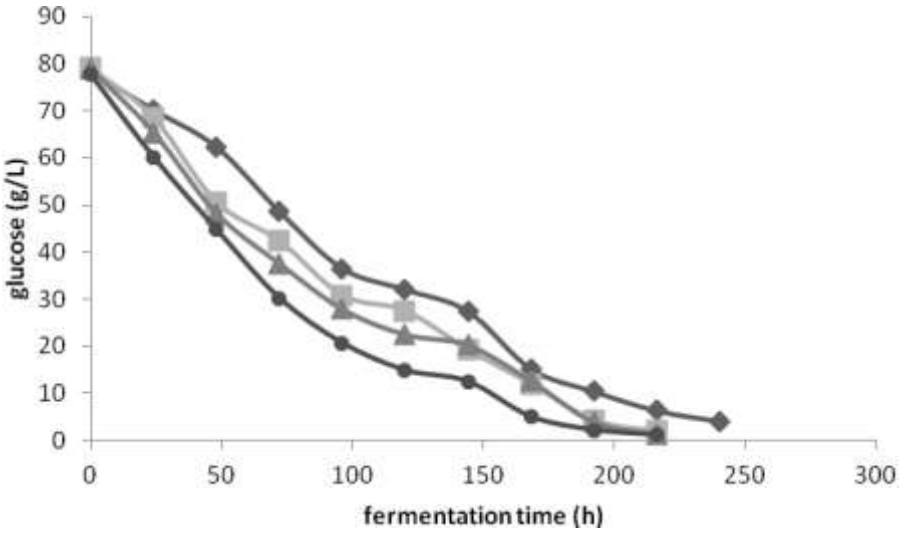

biomass (g/L)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0
50
100
150
200
250
300
fermentation time (h)

Fig. 2


glucose (g/L)
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
fermentation time (h)

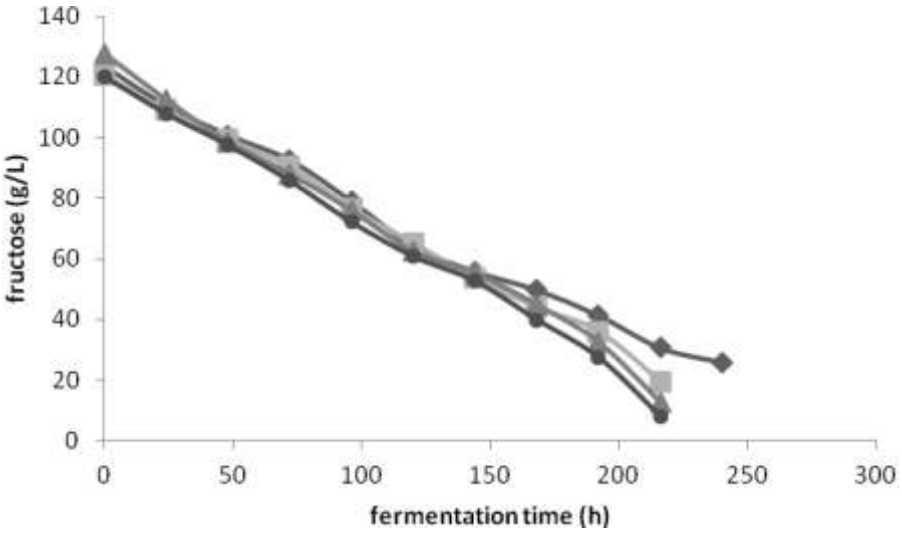

fructose (g/L)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
fermentation time (h)

Fig. 3 a,b

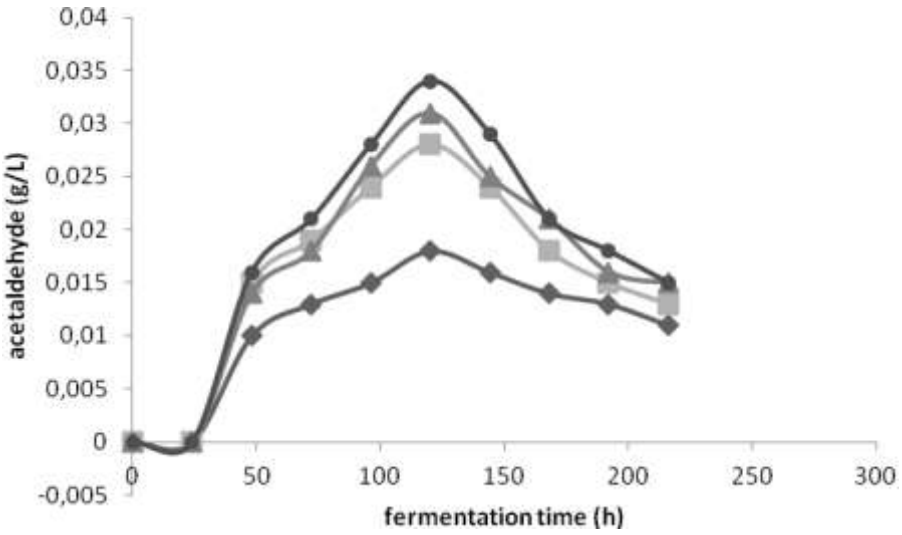
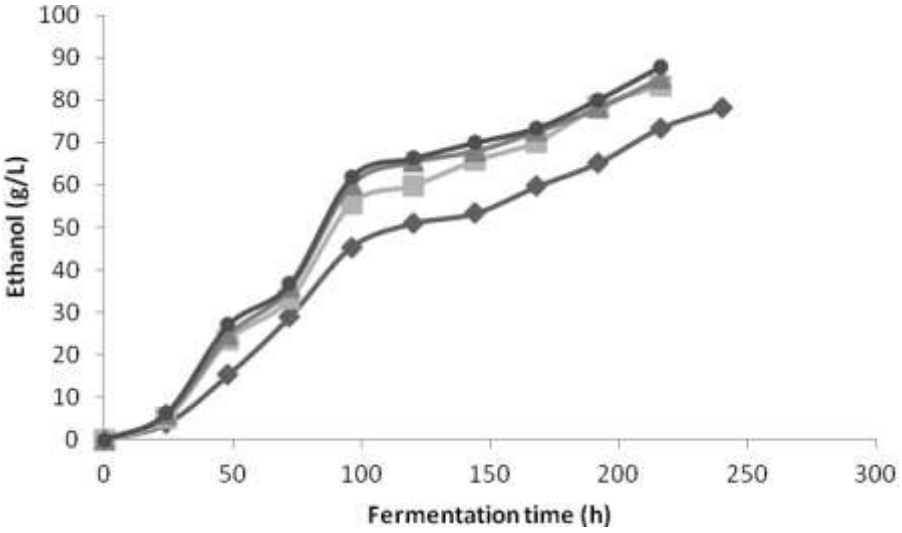

0,04
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
-0,005
acetaldehyde (g/L)
0
50
100
150
200
250
300
fermentation time (h)

Fig. 4


Ethanol (g/L)
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Fermentation time (h)

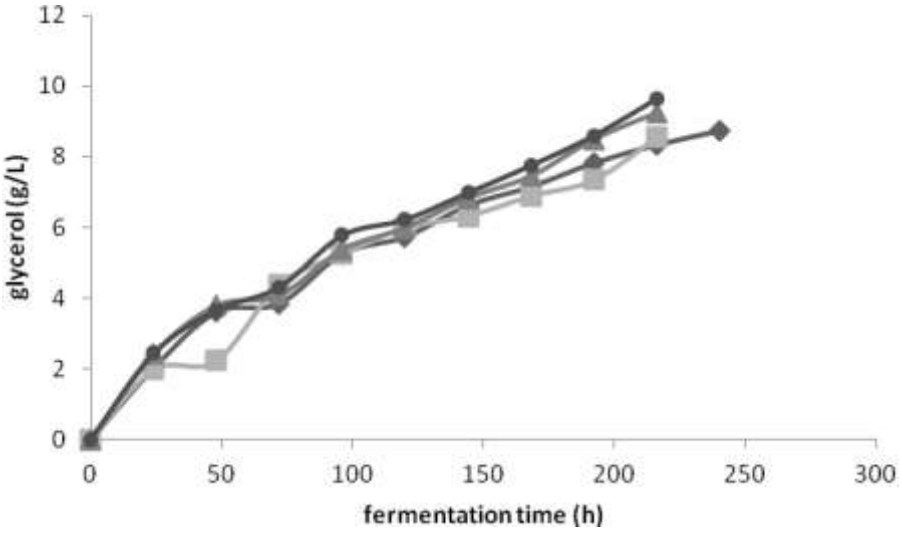
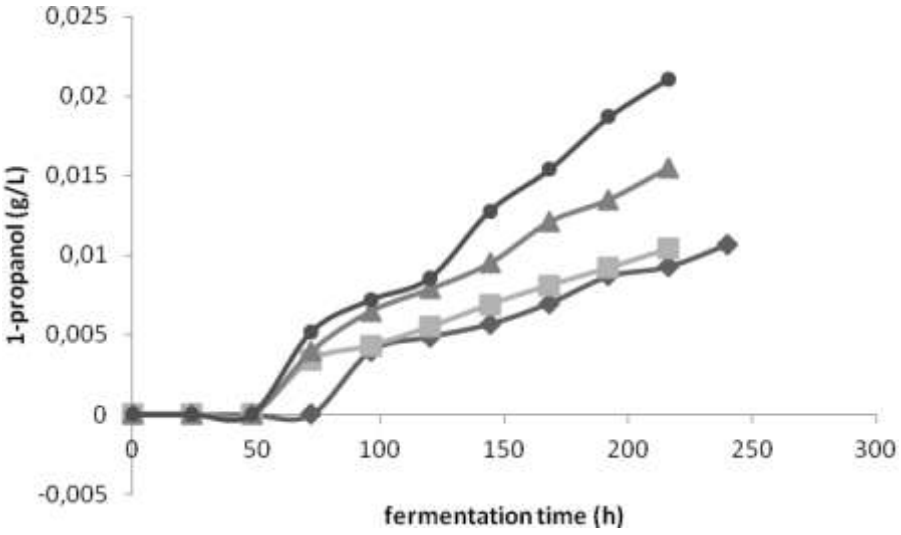
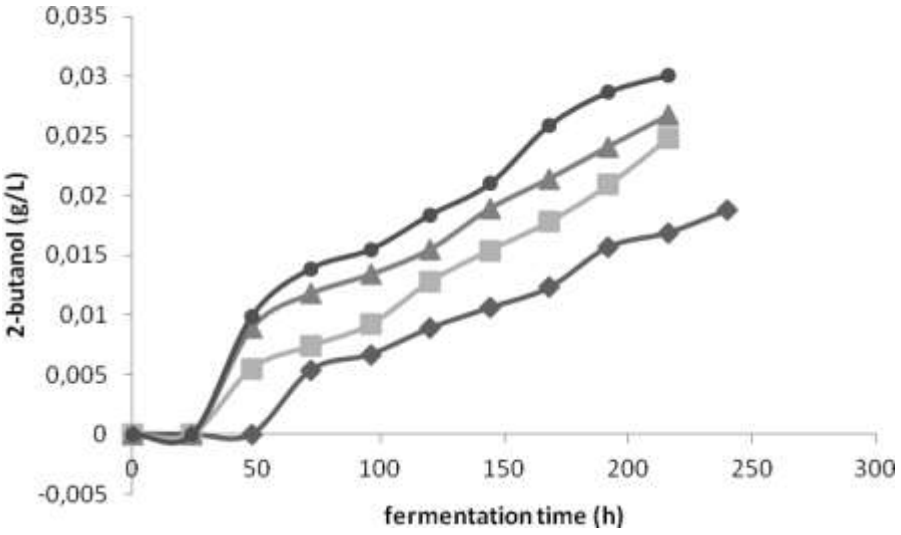

glycerol (g/L)
12
10
8
6
4
2
0
0
50
100
150
200
250
300
fermentation time (h)

Fig. 5 a,b


0,025
0,02
0,015
0,01
0,005
0
-0,005
1-propanol (g/L)
0
50
100
150
200
250
300
fermentation time (h)


0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
-0,005
2-butanol (g/L)
0
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150
200
250
300
fermentation time (h)

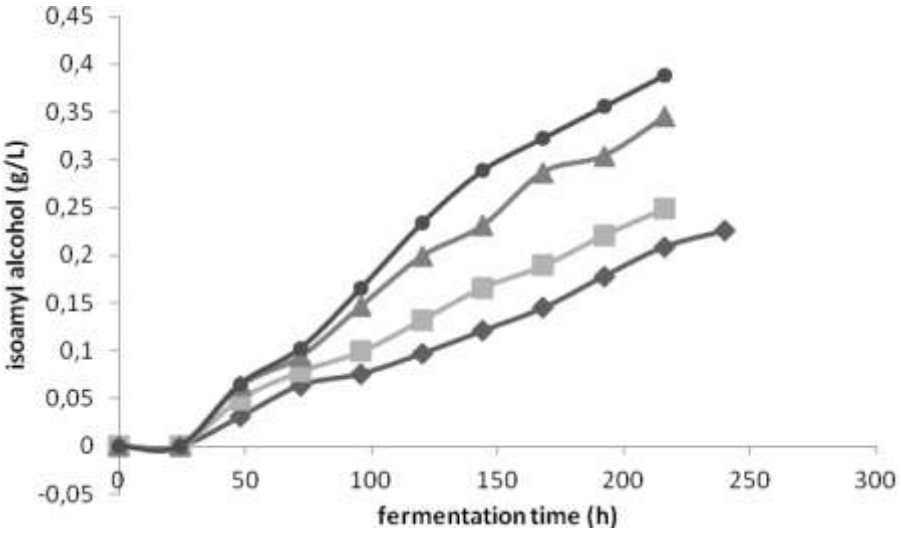
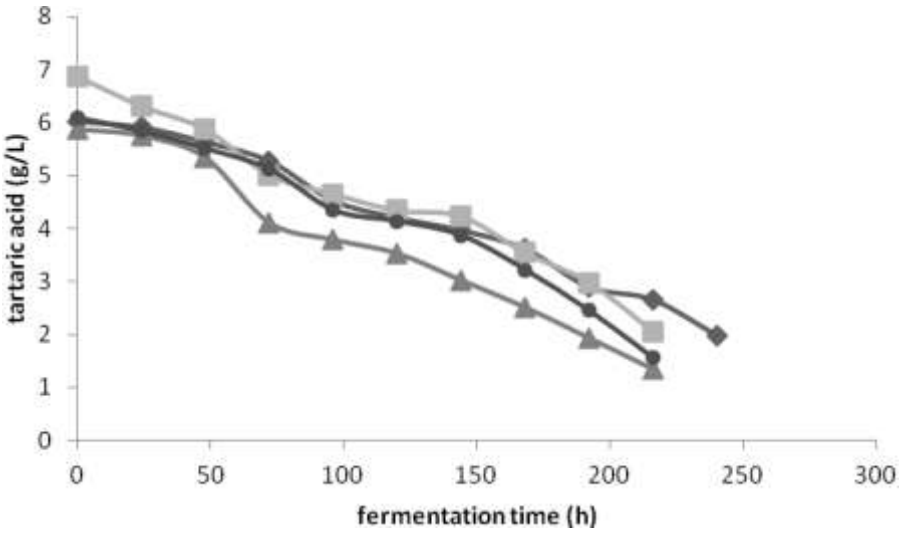
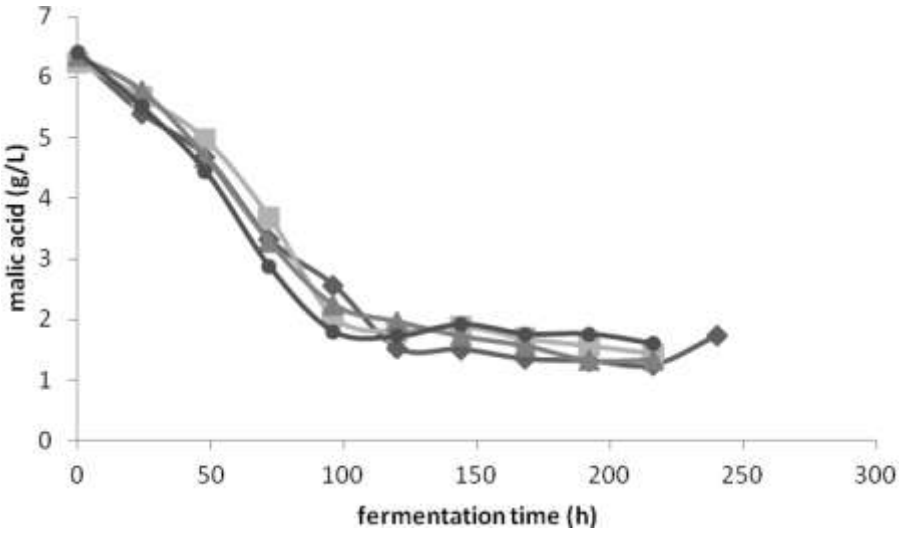

0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
-0,05
isoamyl alcohol (g/L)
0
50
100
150
200
250
300
fermentation time (h)

Fig. 6 a,b,c


tartaric acid (g/L)
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
300
fermentation time (h)


malic acid (g/L)
0
1
2
3
4
5
6
7
0
50
100
150
200
250
300
fermentation time (h)

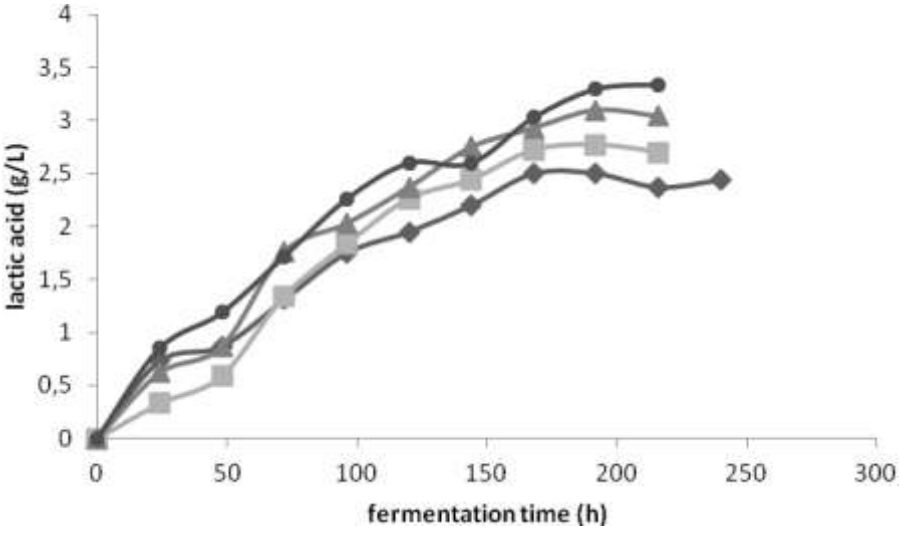

lactic acid (g/L)
0
0,5
1
1,5
2
2,5
3
3,5
4
0
50
100
150
200
250
300
fermentation time (h)

Fig. 7 a,b,c

Table 1

Exposition time (hours)	**24**	**48**	**72**
Control (cells/ml)	5,34 *10 ^{6}	6,36 *10 ^{7}	7,1 *10 ^{7}
Exposed cells (cells/ml)	6,86 *10 ^{6}	8,34 *10 ^{7}	9,27 *10 ^{7}
Cell multiplication increase (**%**)	28.5	27.3	30.0