New Aspects in Wine Technology and Process Engineering

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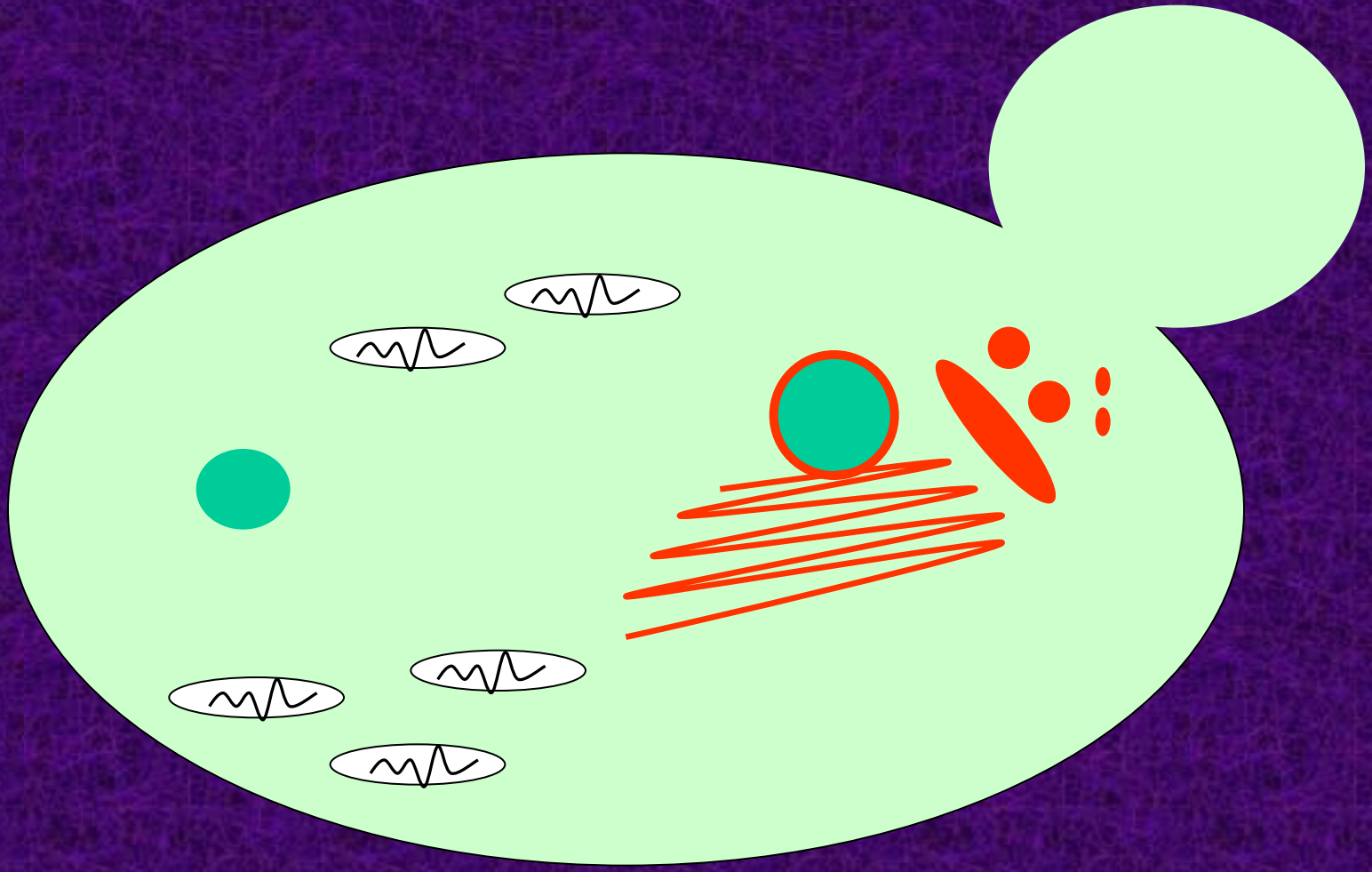
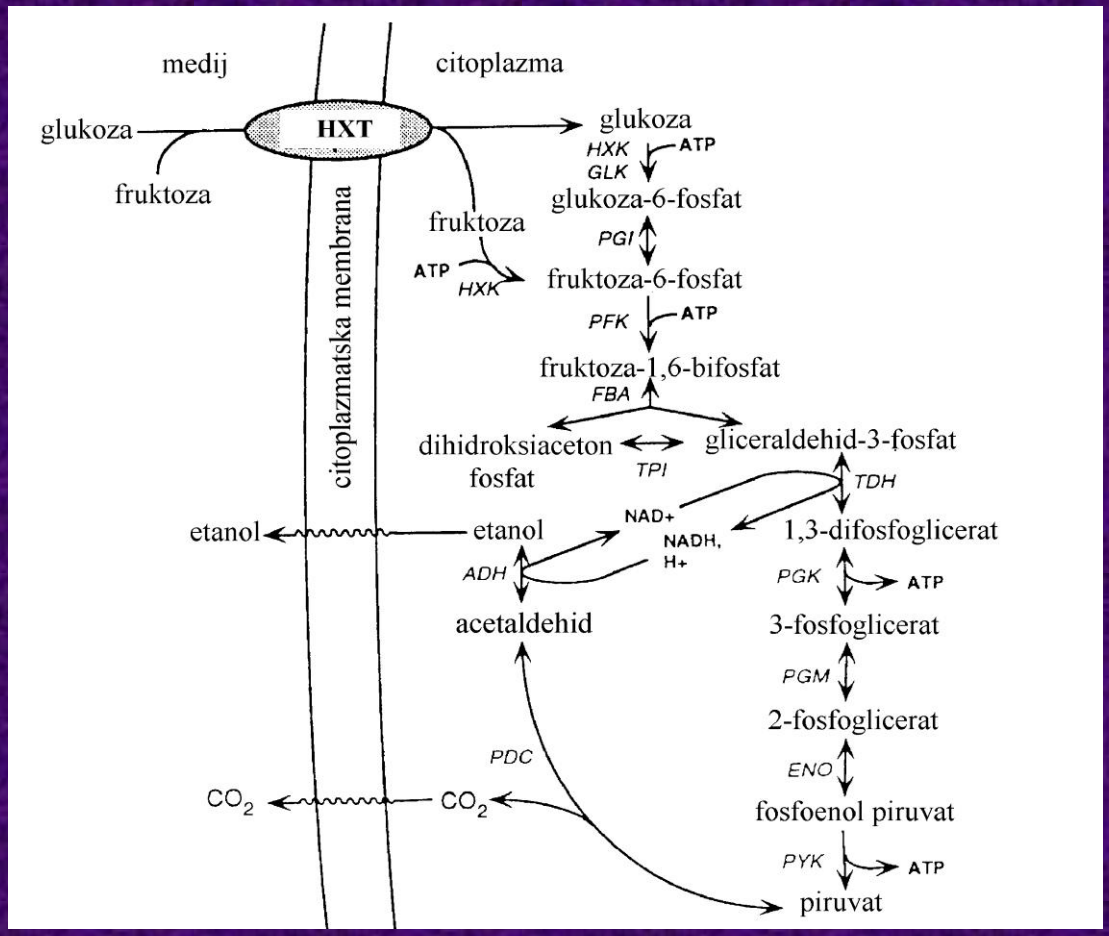
Stirred Tank Reactor
and instrumentation for *on-line* process control

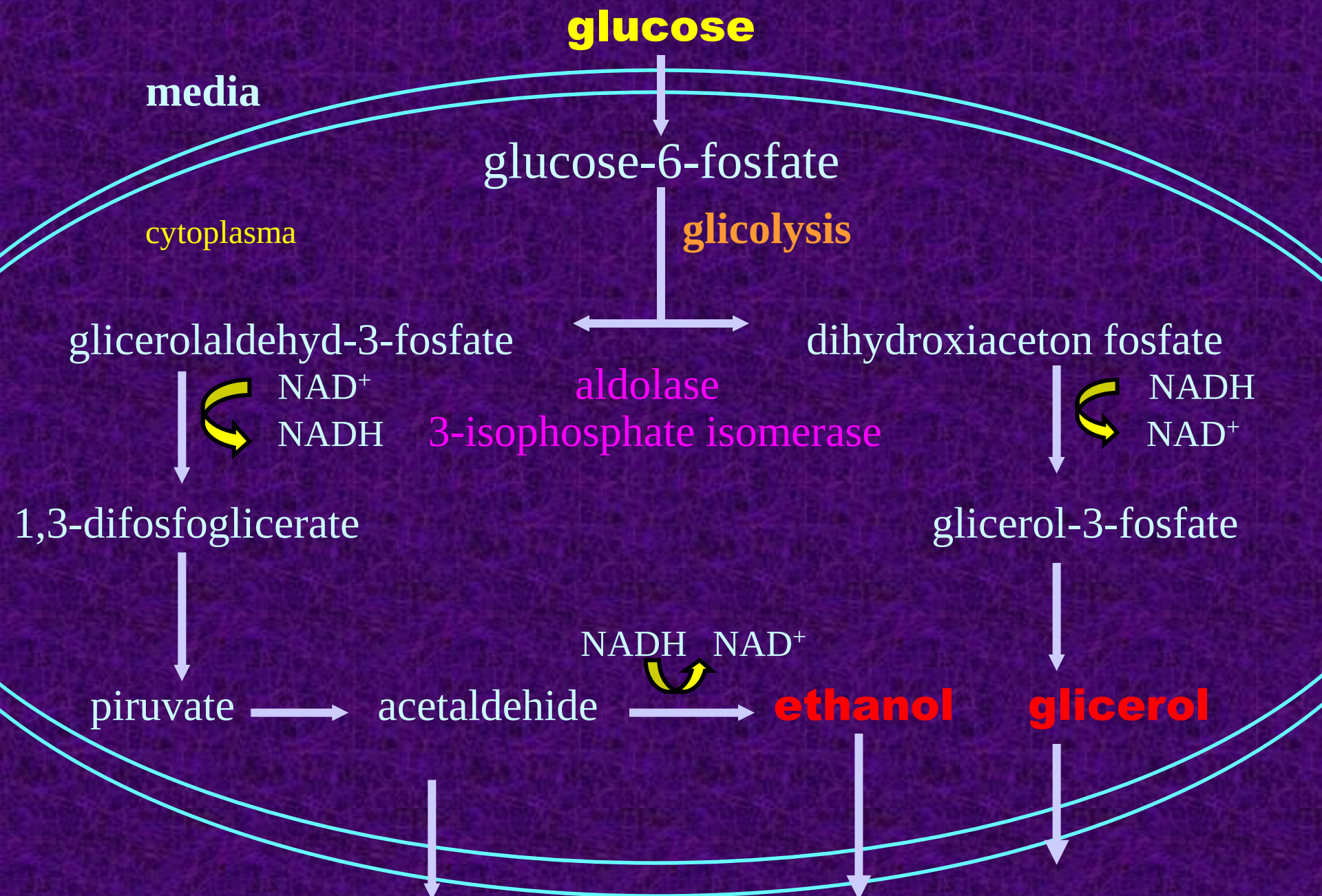
Metabolism of *Saccharomyces cerevisiae*



ALCOHOL FERMENTATION



Metabolism of *Saccharomyces cerevisiae*



Methods and possibilities for *on-line* control of metabolism of *Saccharomyces cerevisiae*

Redox Potential in Wine Technology

- BEROVIC, Marin, MAVRI, Jan, WONDRA, Mojmir, KOSMERL, Tatjana, BAVČAR, Dejan. Influence of temperature and carbon dioxide on fermentation of Cabernet Sauvignon must.
  Food technol. biotechnol., 2003, vol. 41, no. 4, 353-359

- PIVEC, Aleksandra, BEROVIC, Marin, CELAN, Stefan, WONDRA, Mojmir. The role of on-line redox potential measurement in Sauvignon blanc fermentation.
  Food technol. biotechnol., 2002, vol. 40, no. 1, 49-55.

- PIVEC, Aleksandra, BEROVIC, Marin, WONDRA, Mojmir, CELAN, Štefan, KOŠMERL, Tatjana. Influence of temperature shock on the glycerol production in cv. Sauvignon blanc fermentation.
  Vitis, 2003, vol. 42, no. 4, 205-206

REDOX POTENTIAL

$$E = E_{calomel} + RT/nF \ln a_{oxidants} / a_{reductants}$$

$$E_{calomel} = 244\ mV\ E_0$$

Standard redox potencial

$$E_h = E_0 + RT/nF \ln a_{oxidants} / a_{reductants}$$

Clark rH potential definition

$$rH = 0.0343\ E_h + 2\ pH\ ;\ rH = -\log pH_2\ ;\ pH = -\log aH_2$$

Redox potential in more complex systems

$$E_h = E^o - \frac{RT}{zF} \ln \left(\frac{\prod_i a_{red}^{\nu_{red}}}{\prod_i a_{ox}^{\nu_{ox}}} \right)$$

$$\nu_1 S_1 + \cdots + \nu_i S_i + ze^- \Leftrightarrow \nu_j S_j + \cdots + \nu_k S_k$$

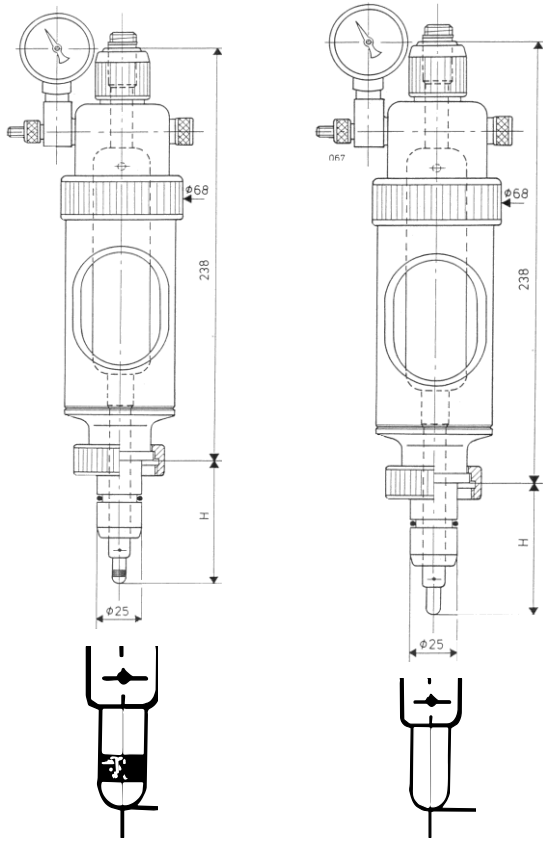
Product of activities of all the oxidants or reductants in the system

$$\prod_i a_i^{\nu_i} \equiv a_{S_1}^{\nu_1} \cdot a_{S_2}^{\nu_2} \cdots a_{S_i}^{\nu_i}$$

$$\prod a^{\nu i}_{\text{oxidants / reductants}}$$

Redox electrode pH electrode

Pt / calomel **glass/calomel**



REDOX POTENTIAL in WINE

Wine represent complex oxydo-reductive system composed by :

→ Chemical oxydo – reducitive compounds :

alcohols, aldehydes, organic (oxo & keto) acids polyphenols, kinons, ascorbic & dehydroascorbic acid,

→Ion couples Fe^{2+}/Fe^{3+} ; Cu^{+}/Cu^{2+}

Redox potential in wine express intensivity and the rate of the sum of all oxydo - reductive processes

Metabolic activity of microbial culture and its metabolism play the main role

→sugars (glucose) → alcohols + biomass

REDOX CHARACTERISTICS of WINES

Premium quality wines are characterized by the lowest redox potential values
Reductive and close character wines are disposed to oxidation

Reductive non-oxydized wines Eh = 100 - 180 mV ; rH=18 do 20
Oxydized wines Eh = 268 - 300 mV ; rH=24 do 25

White bottled wines : Eh = 275 -280 mV
Red bottled wines : Eh = 100 -223 mV

REDUCTANTS

SO2, ascorbic acid, cell products (cisteine , glutatione) are parameters that are decreasing redox potential and express reductive character

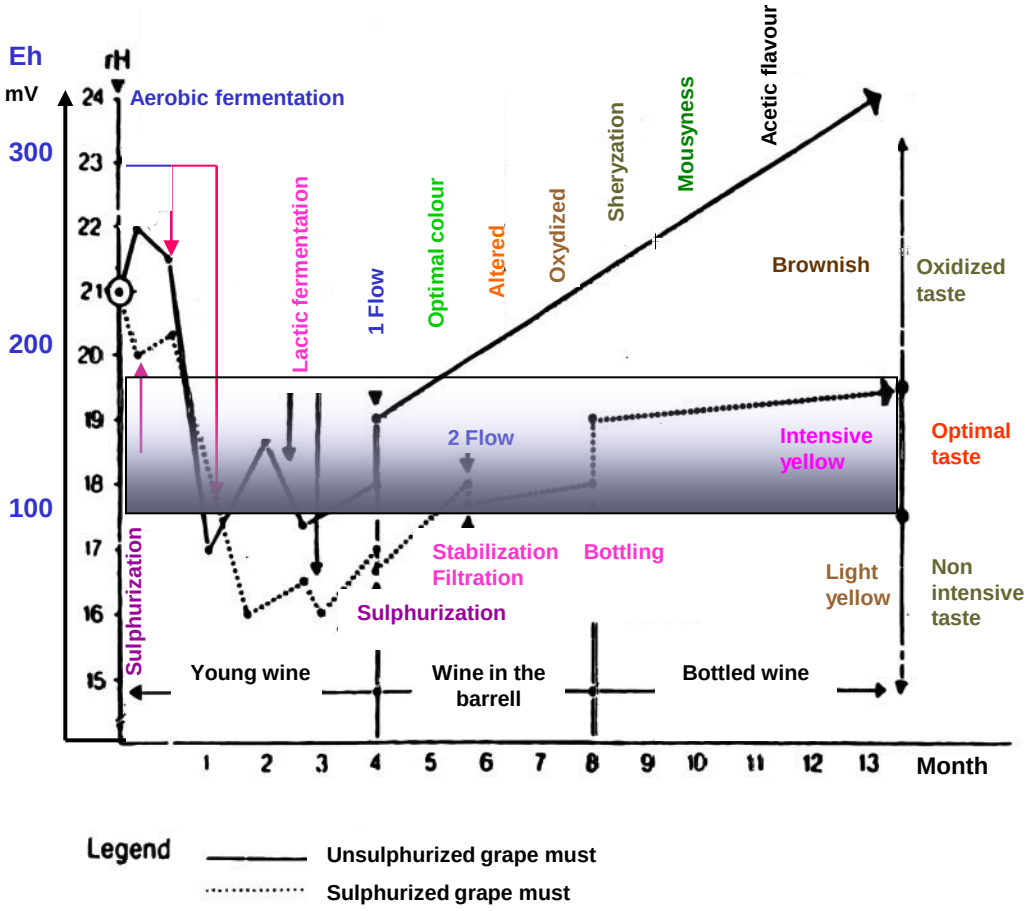
OXIDANTS

Air oxygen, bentonites, mineral adsorbents, peroxyde

PROTECTORS AND ANTIOXIDANTS IN FERMENTATION

Carbone dioxyde, gaseous nitrogen are protective agents
in the pipes and fermentor head space

Redox potential and sensorial analysis in wine fermentation



Reductive non-oxydized wines Eh = 100 - 180 mV ; rH=18 do 20 Oxydized wines Eh = 268 - 300 mV ; rH=24 do 25

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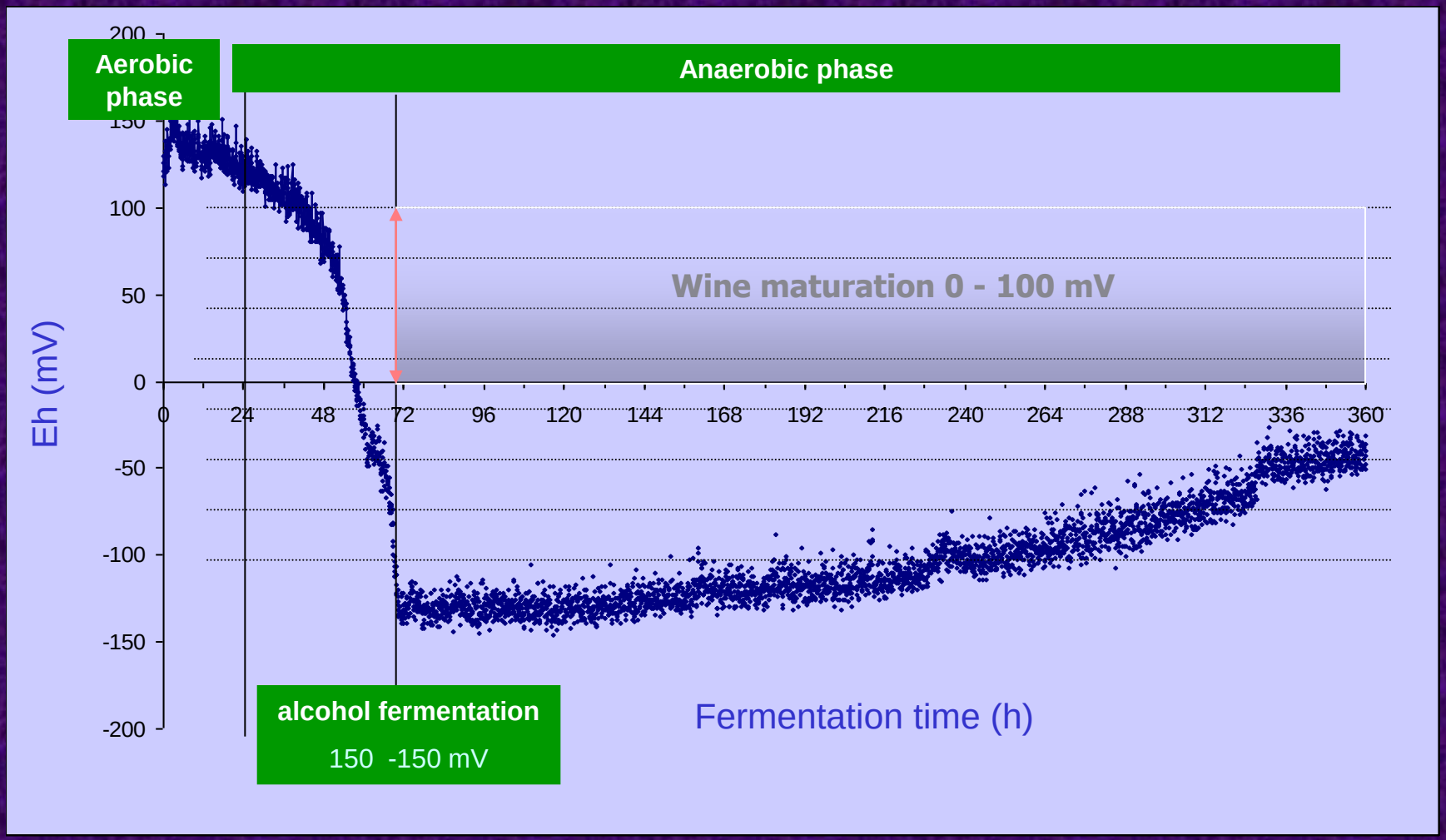
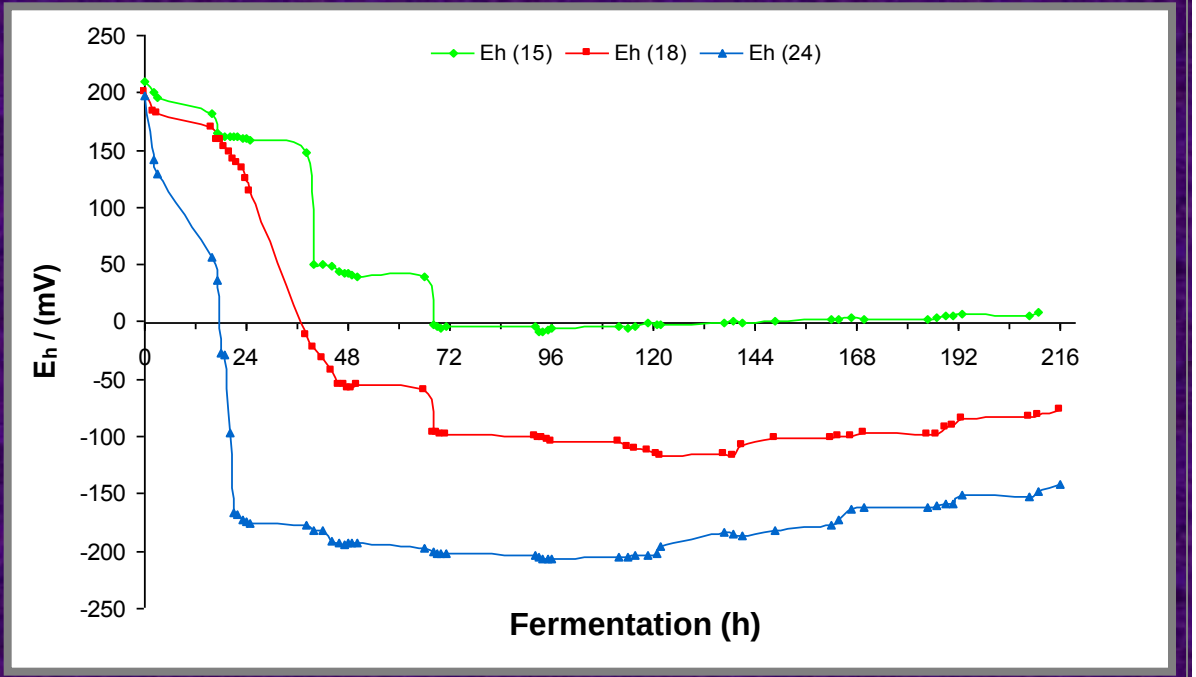
Redox potential of unsulfurized *Sauvignon blanc* must alcohol fermentation



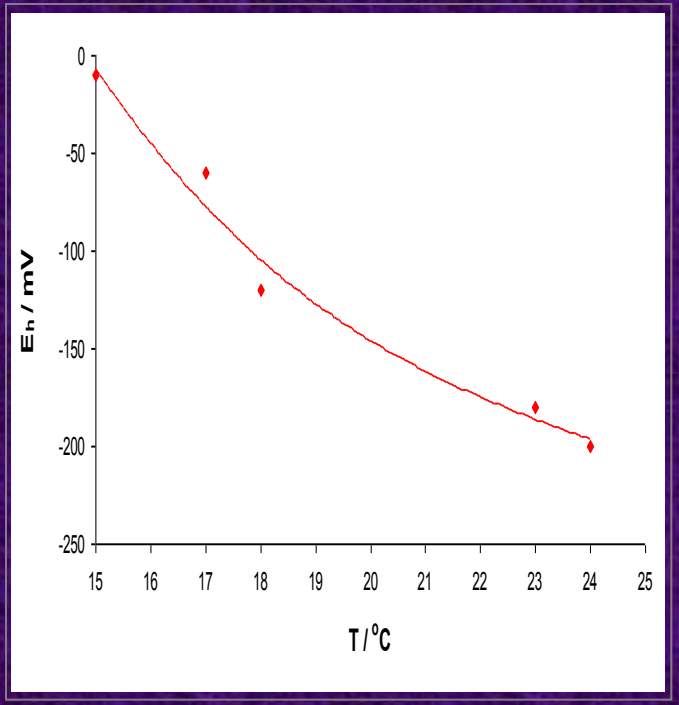
Fermentation temperature 18°C

Temperature influence

T [°C]	E_h [mV] - starting	E_h [mV] - final	ΔE_h [mV]
15	210	-10	220
18	200	-150	350
24	200	-200	400

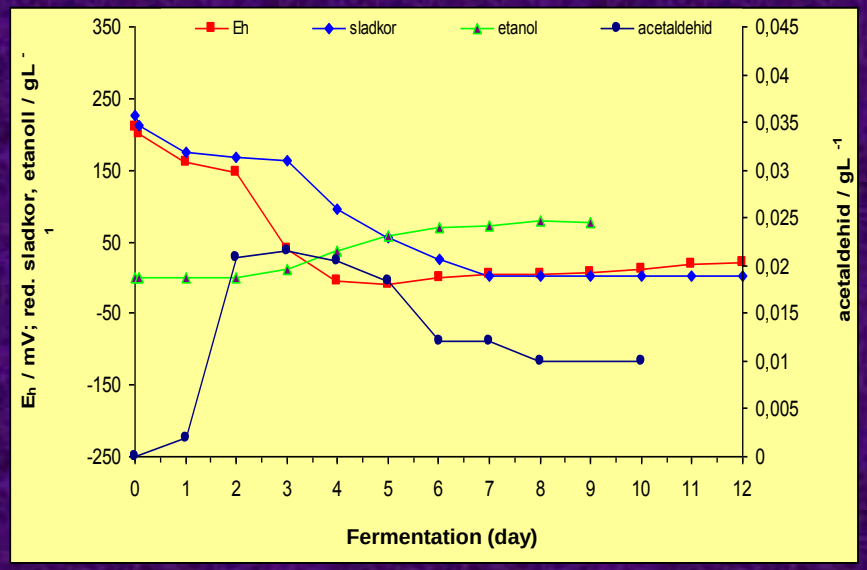


Redox potential at 15, 18 in 24 °C

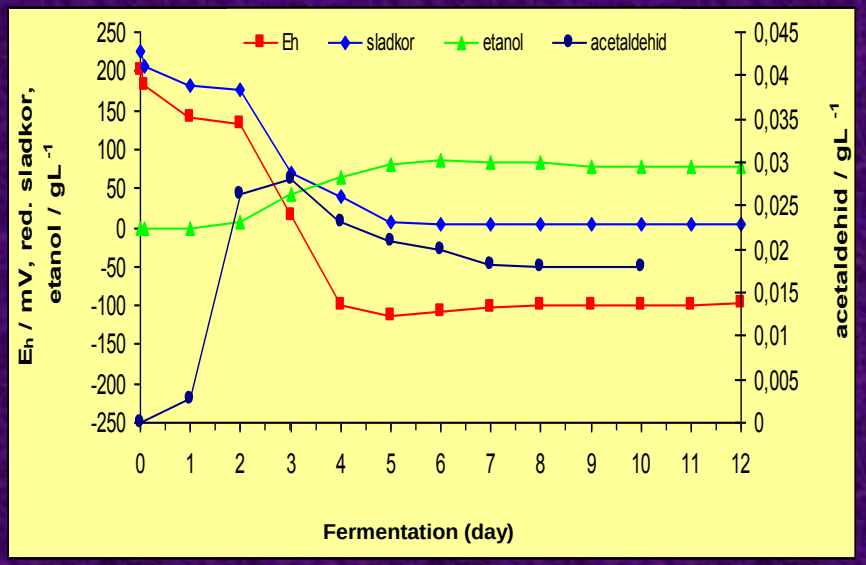


Eh [mV] - final as a function of temperature

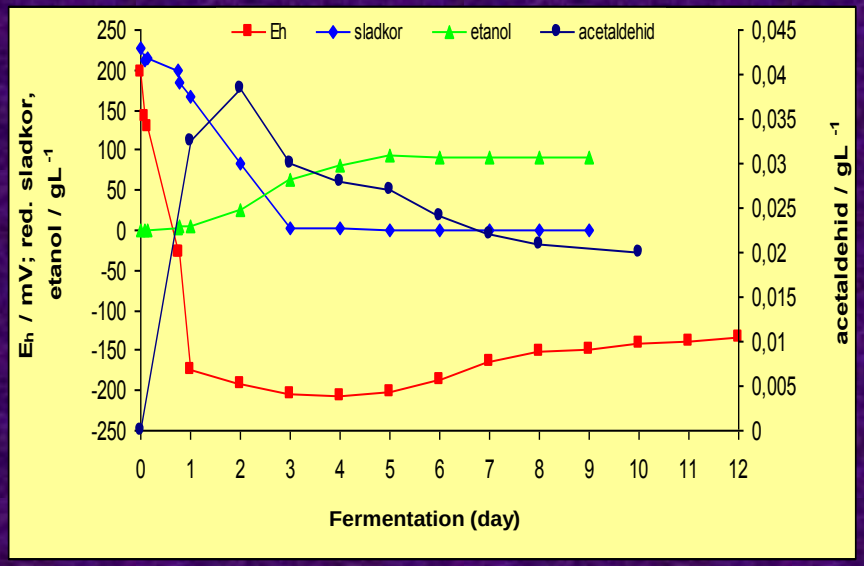
15 ºC



18 ºC

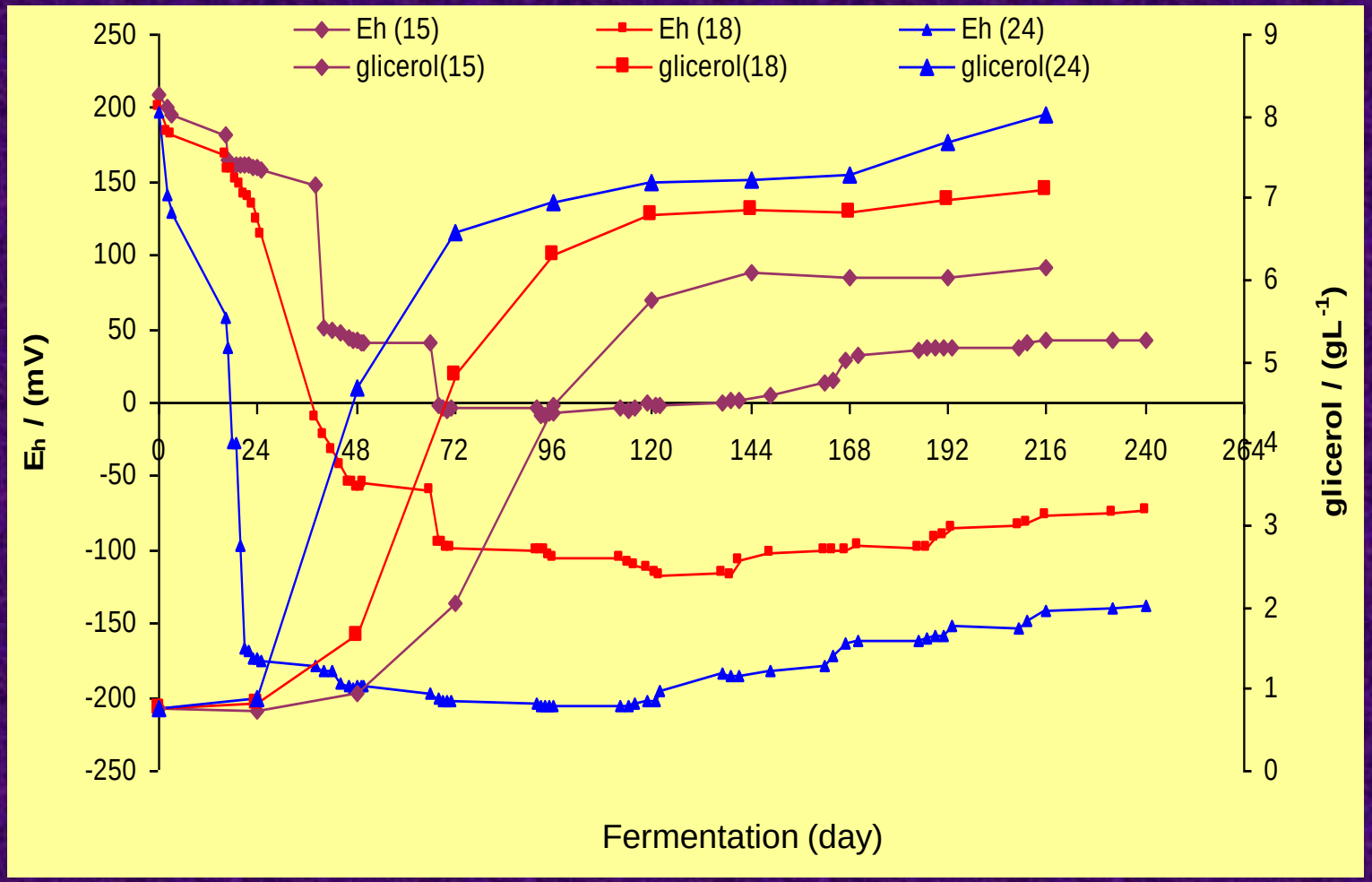


24 ºC



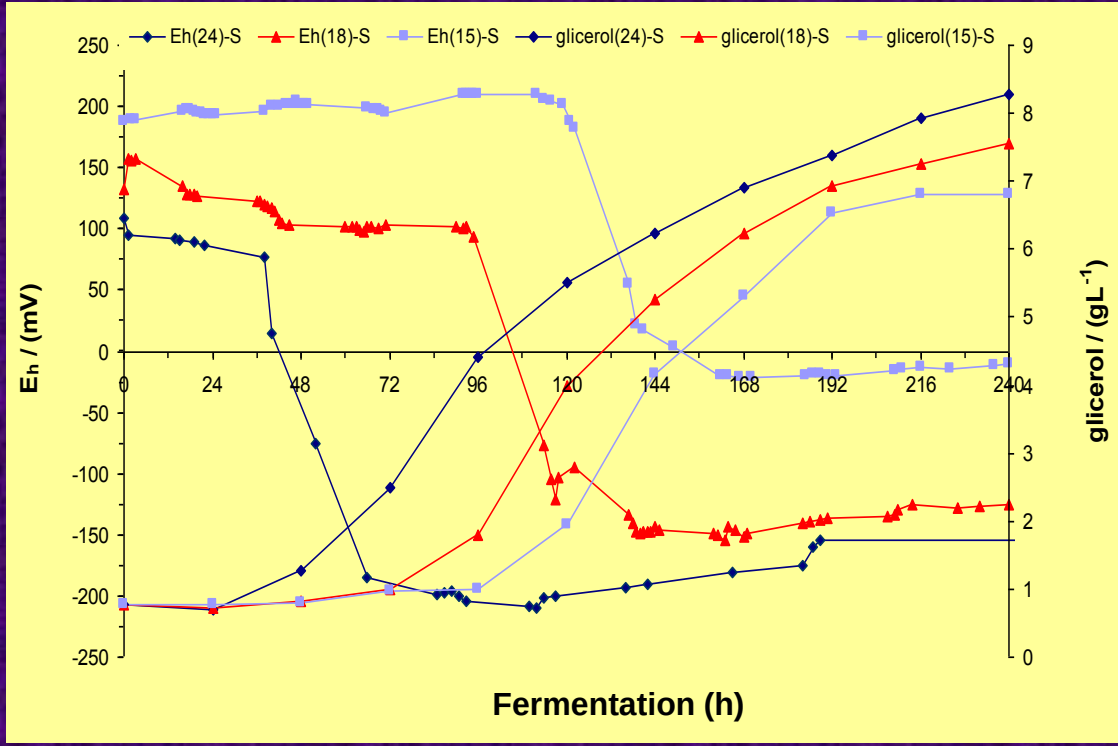
Redox potential, reducitive sugars consumtion, acetaldehyde and ethanol at 15, 18 in 24°C

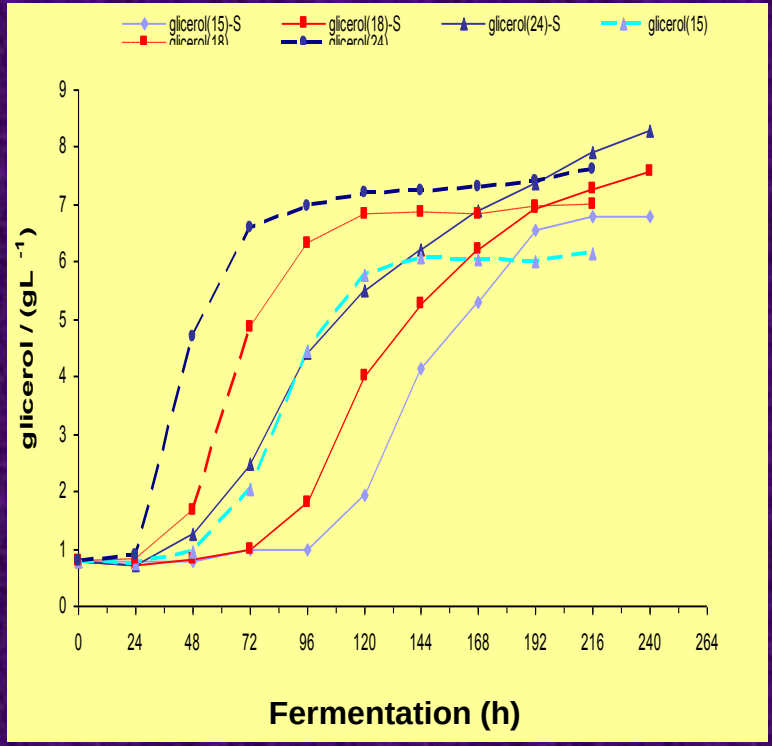
Temperature influence on redox potential and glycerol biosynthesis



Fermentation temperatures 15, 18 and 24 °C

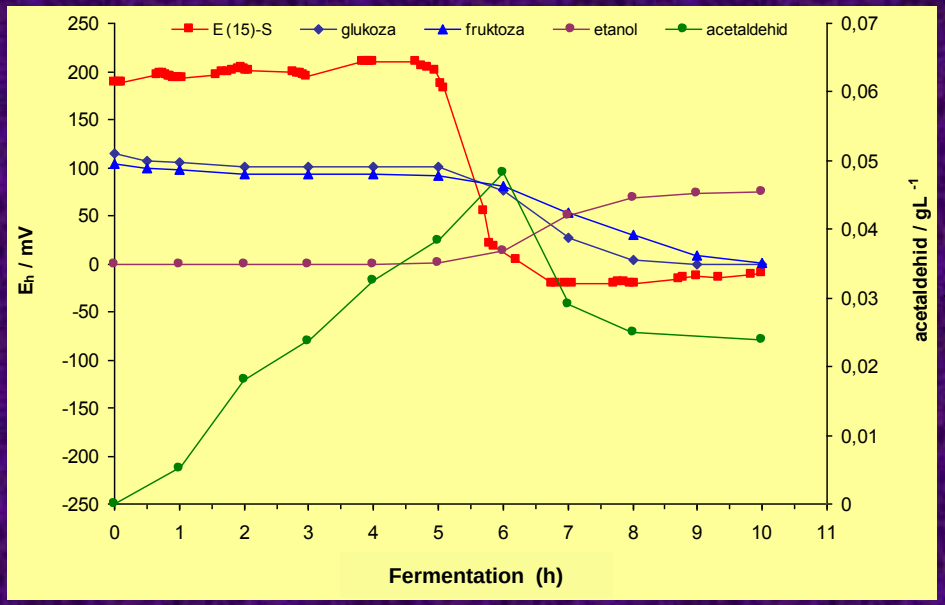
Redox potential and glycerol in sulfurized grape must



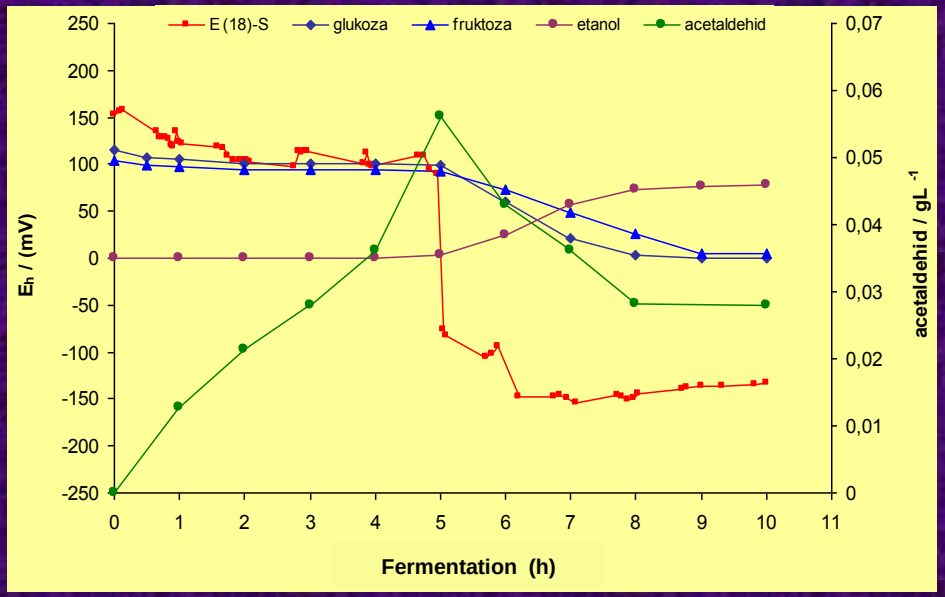


Fermentation temperatures pri 15, 18, 24 °C

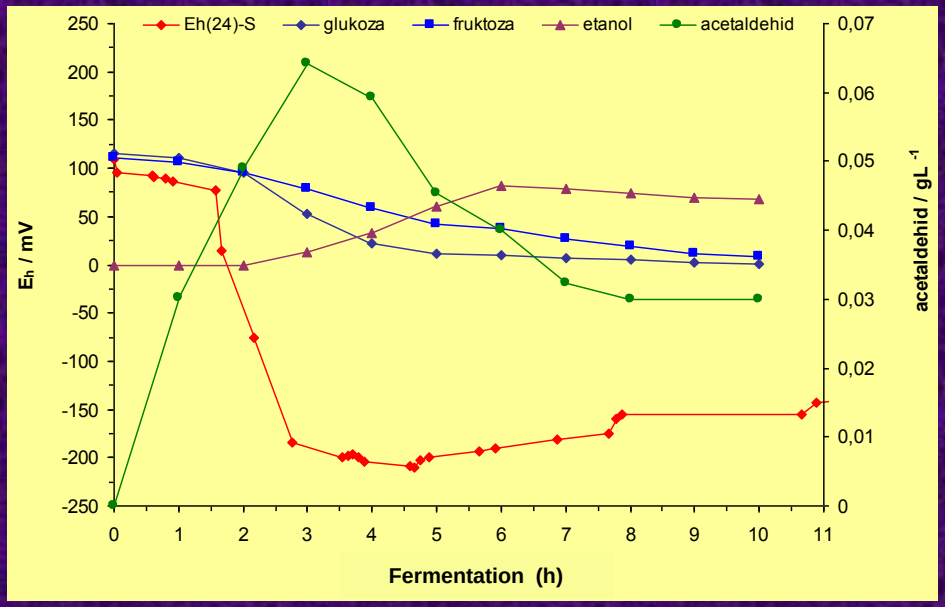
Consumption of glucose and fructose, production of ethanol in sulfurized grape must



15°C



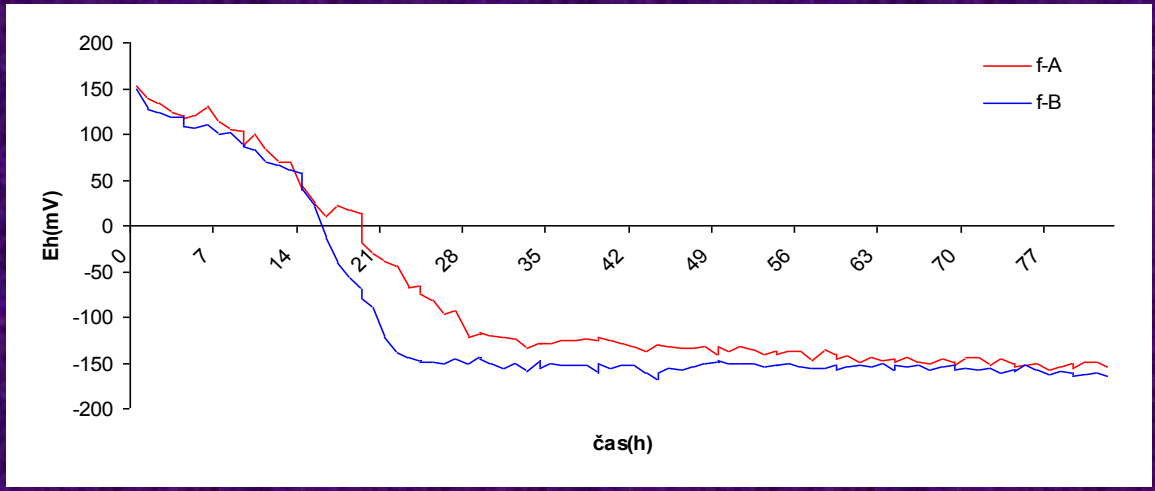
18°C



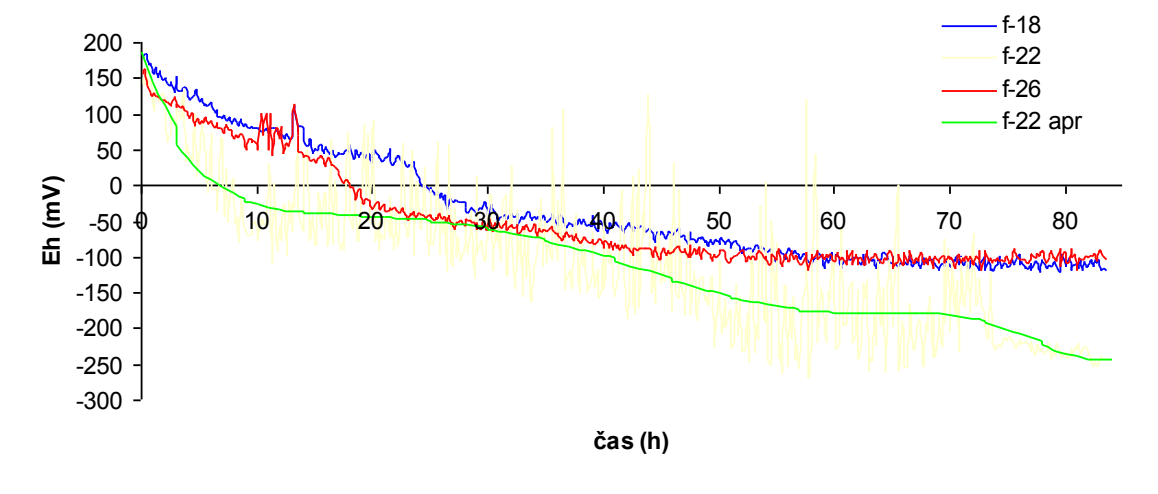
24°C.

Redox potential in other cultivar fermentation

Redox potential in *Cabernet sauvignon* fermentation

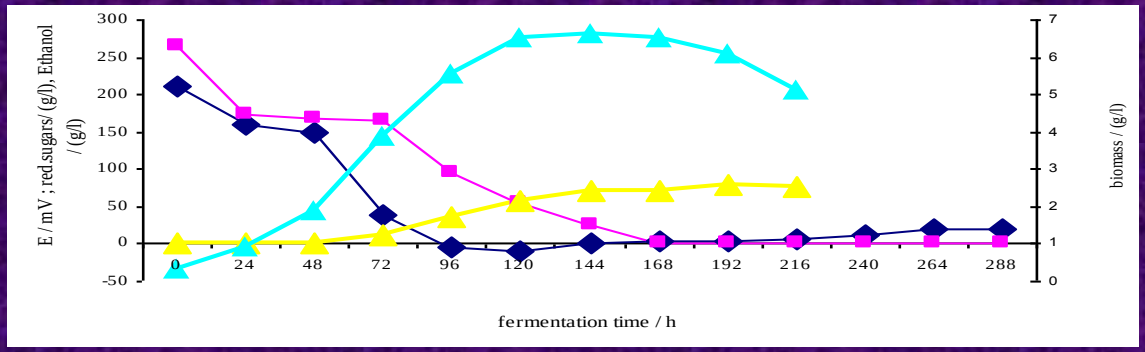


Fermentation with and without additional CO_2

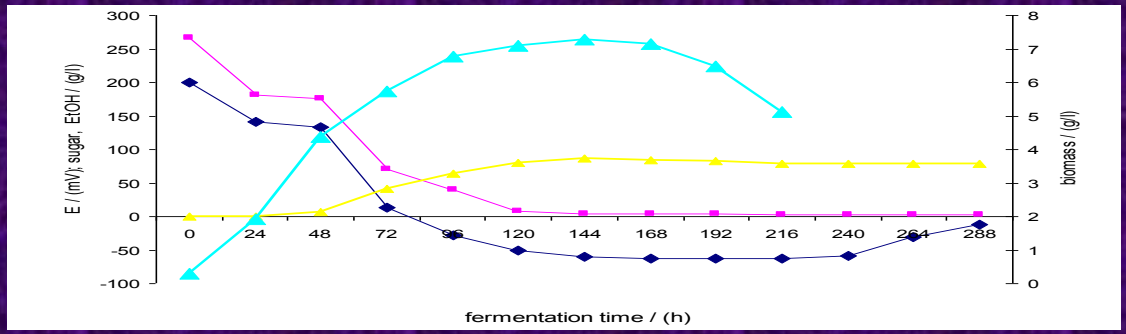


Fermentation with additional CO_2 at T 18 , 22, 26 °

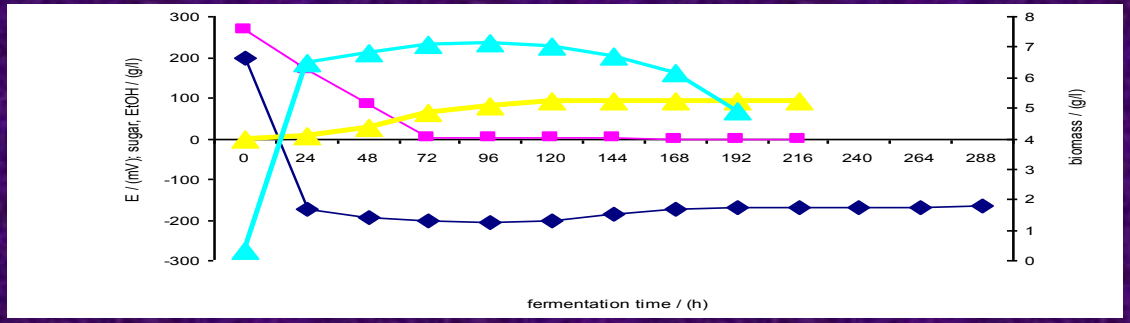
Sauvignon blanc fermentation



T 15°C

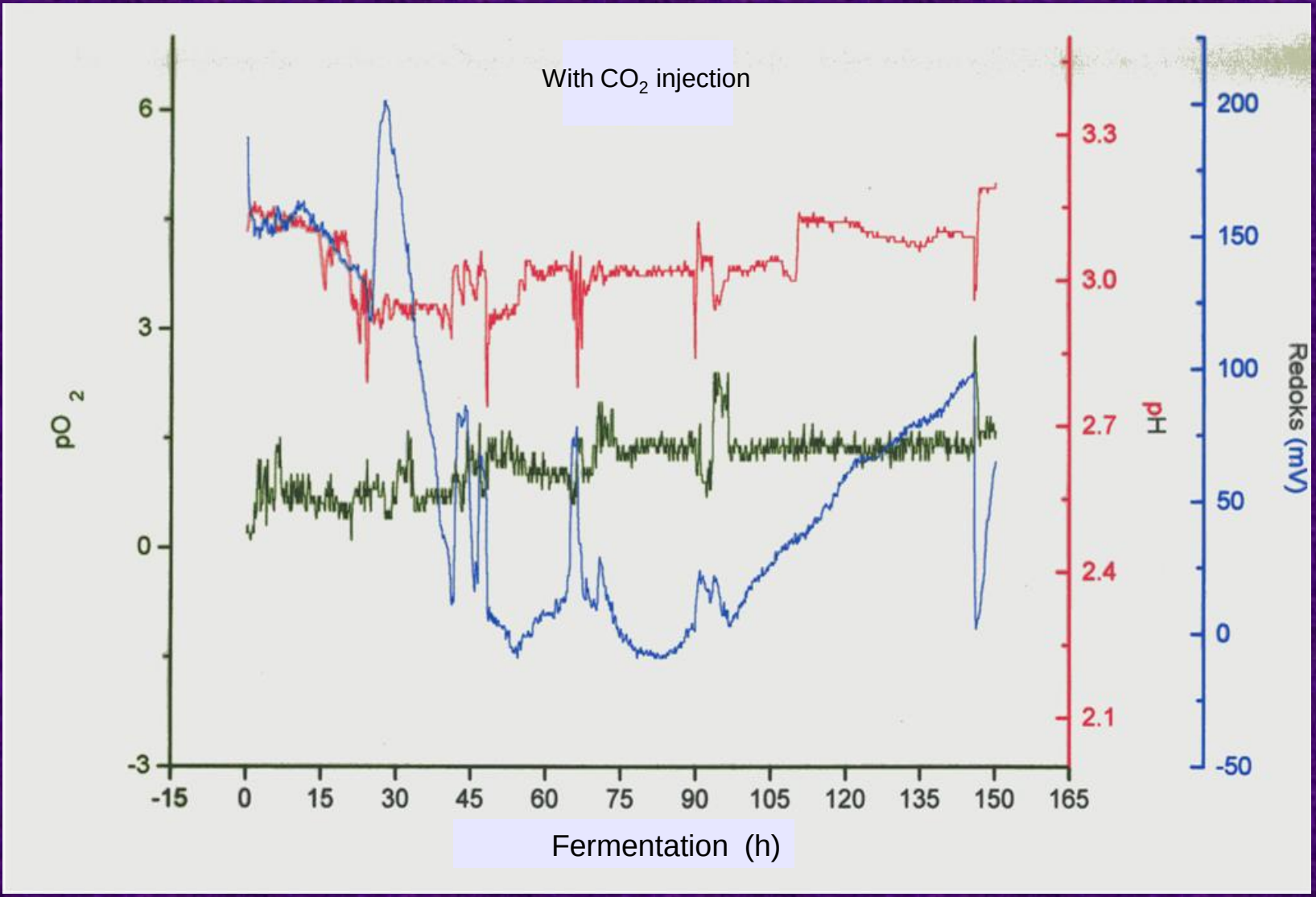


T 18 °C



T 24 °C

Blue frankish fermentation T 24 °C *on-line* data

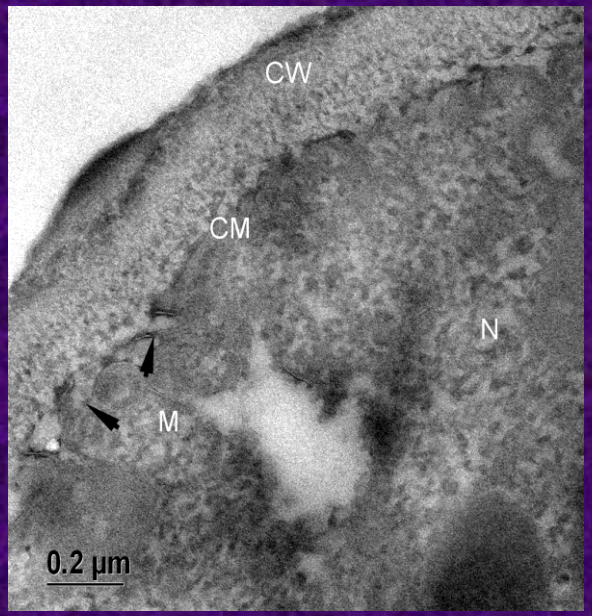


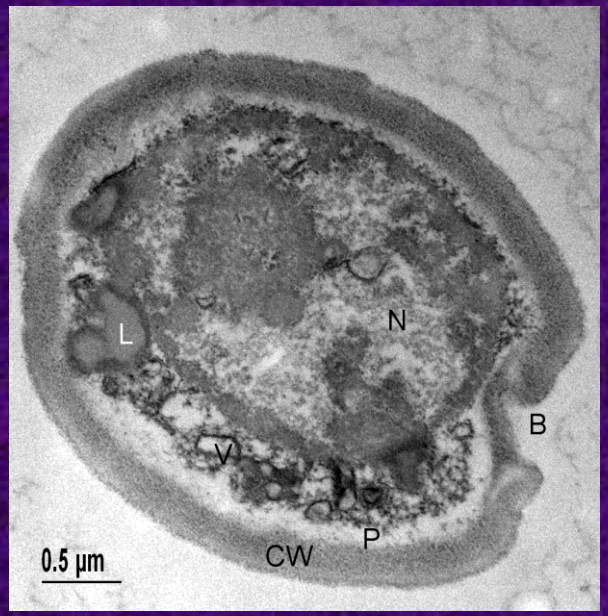
Influencing factors for regulation of *Saccharomyces cerevisiae* metabolism

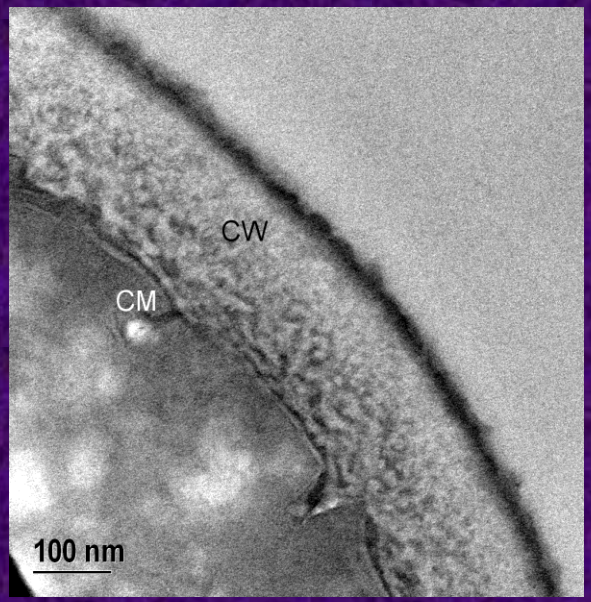
The Influence of Galvanic Field on *Saccharomyces cerevisiae* in Grape Must Fermentation

M. BEROVIC [1)] , M. POTOCNIK [1)] and J. STRUS [2)]

Vitis 47 (2), 117-122 (2008)





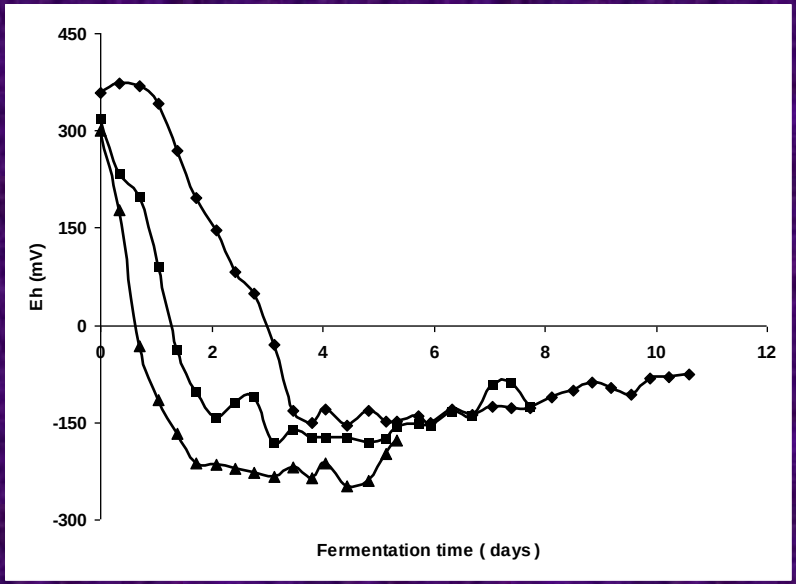


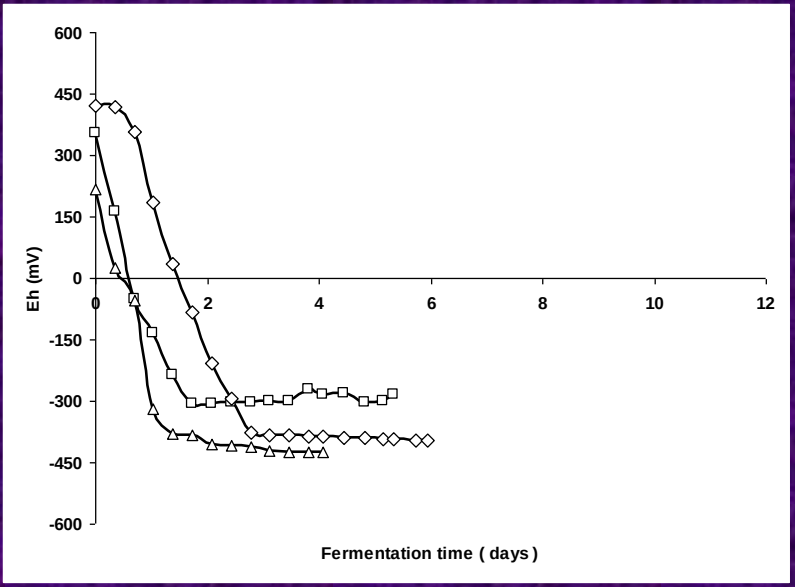
GALVANIC FIELD

S. cerevisiae **in fermentation with electrostimulation**

Current 30 µA , after 72 hours, colourized with methylen blue

The influence of galvanic field on *Saccharomyces cerevisiae* in grape must fermentation



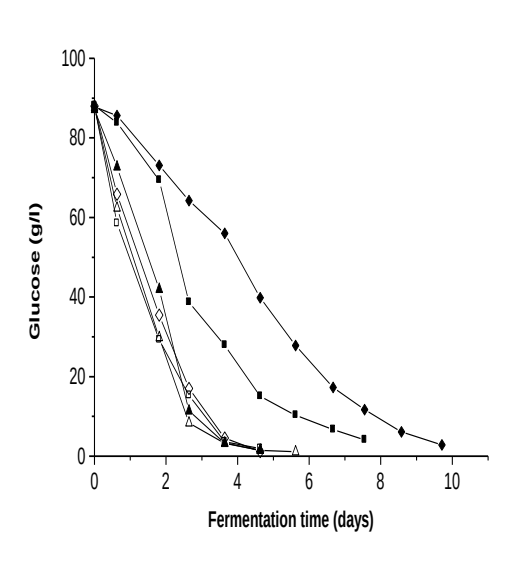


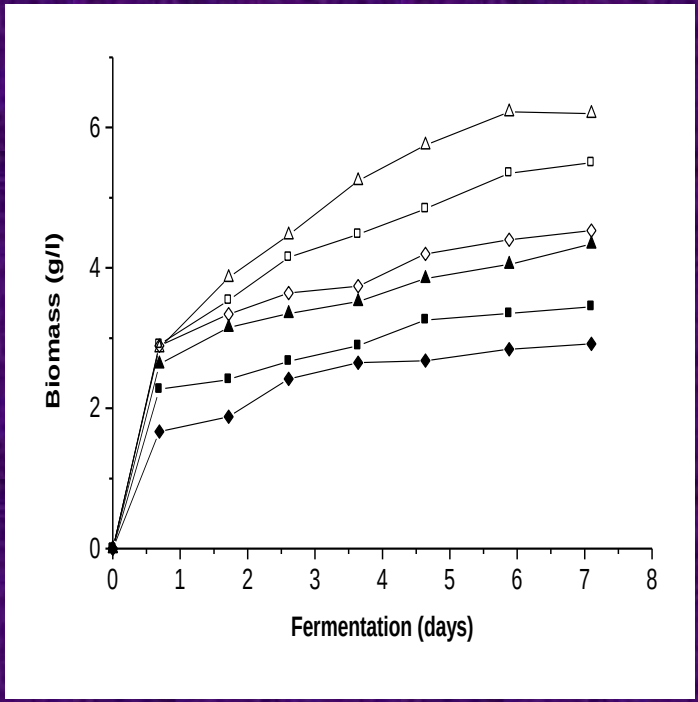
Fermentation *on-line* redox potential courses

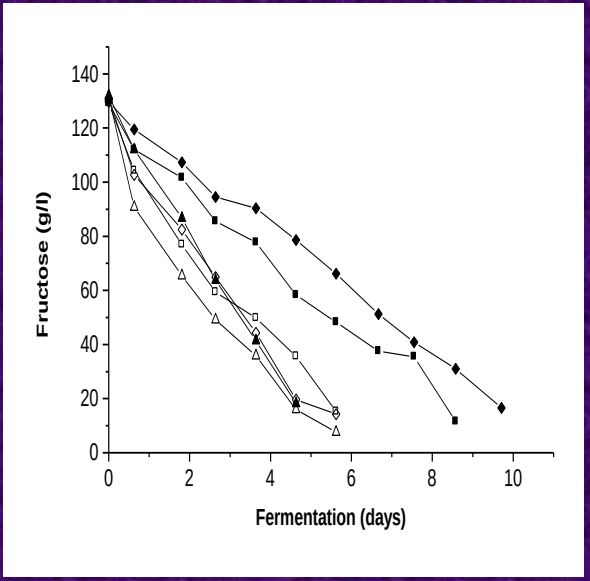
♦T = 18, ■ 22 and ▲ 26 °C , without electrostimulation

◊ T = 26° C (1,3 µA) , □ T = 26° C (7,7 µA), Δ T = 26° C (30 µA), with electrostimulation.

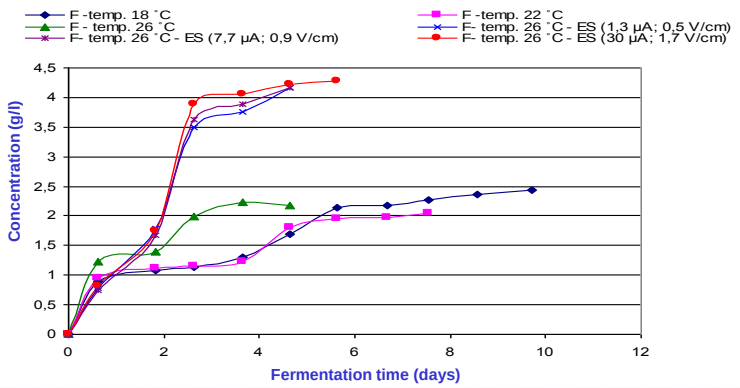
The influence of galvanic field on *Saccharomyces cerevisiae* in grape must fermentation



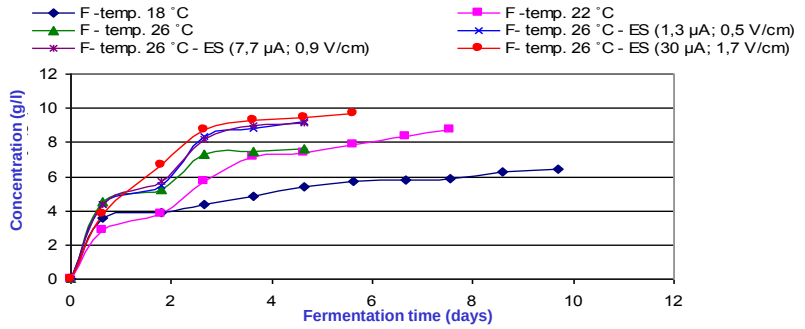




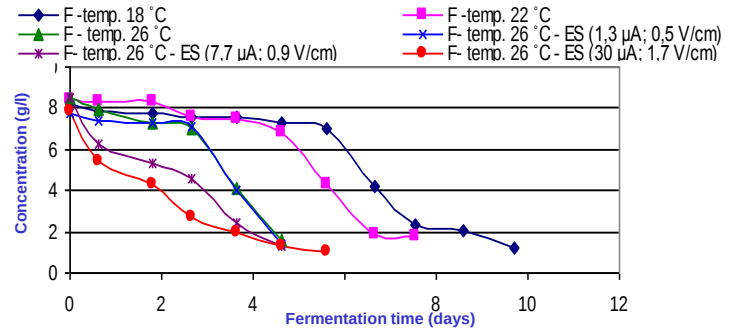
Lactic acid



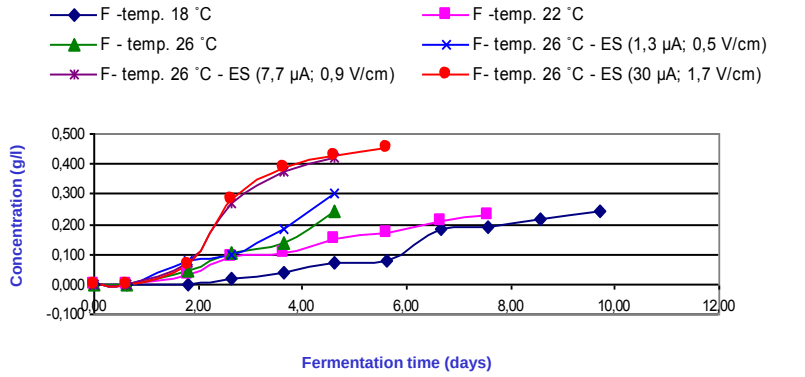
Glycerol



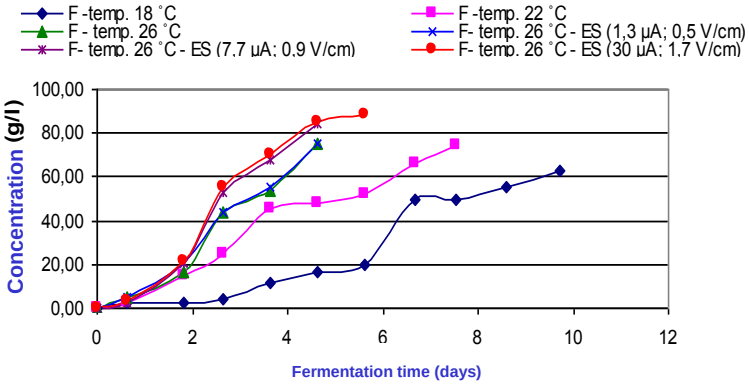
Malic acid



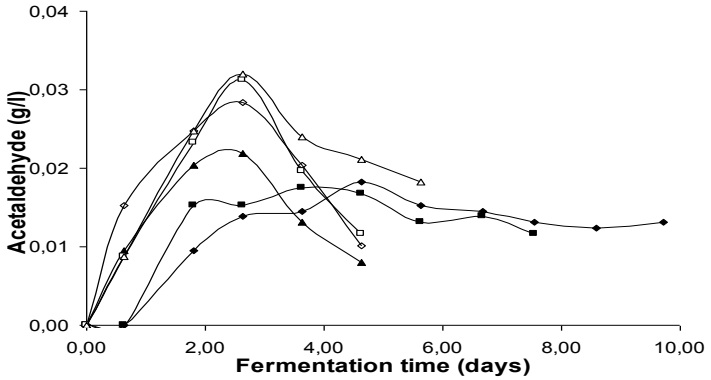
Iso-amilalcohol



Ethanol

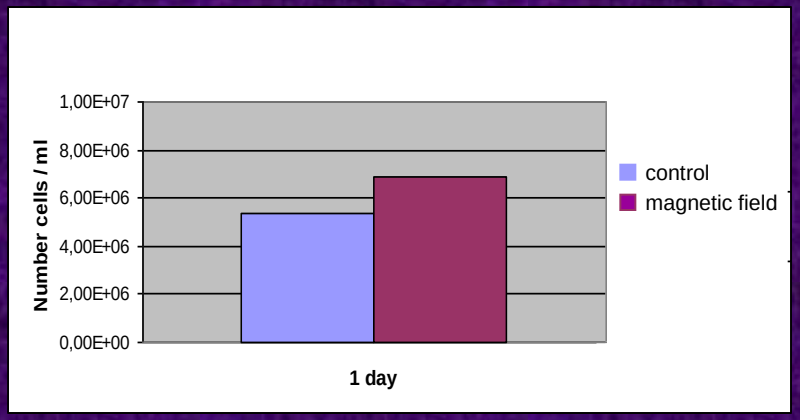


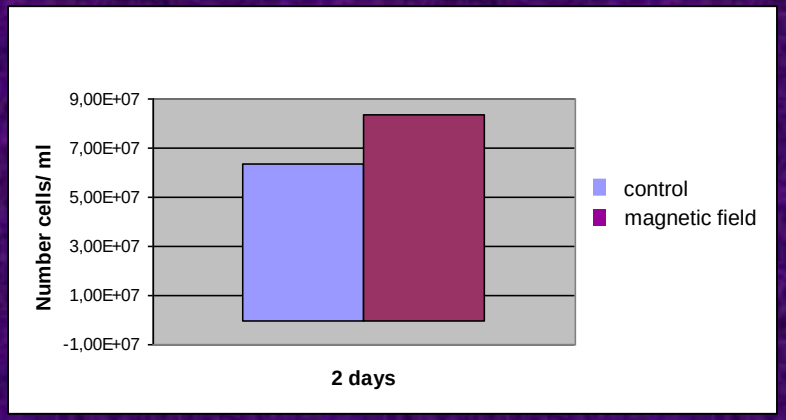
Acetaldehyde

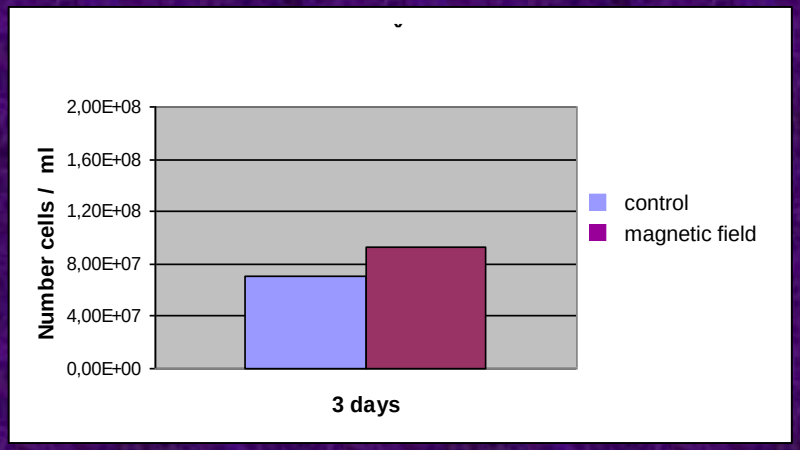


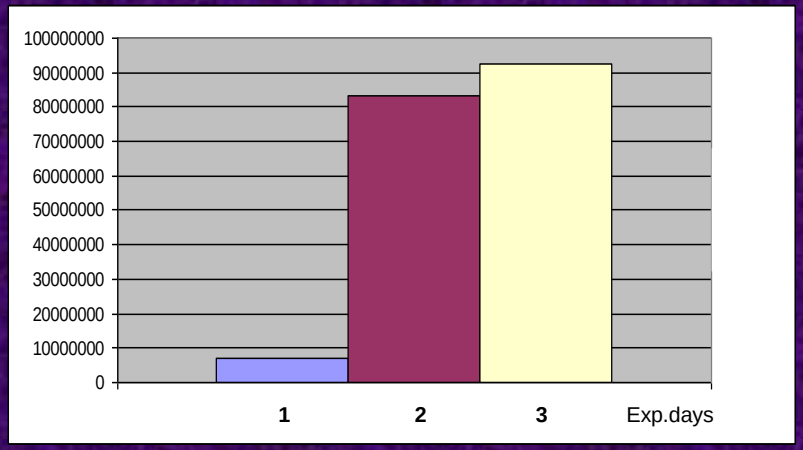
MAGNETISM
&
MAGNETIC FIELD

Influence of static magnetic field on *Saccharomyces cerevisiae* metabolism

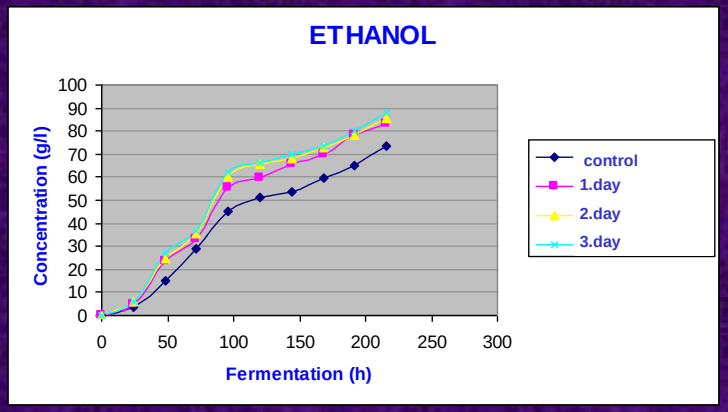


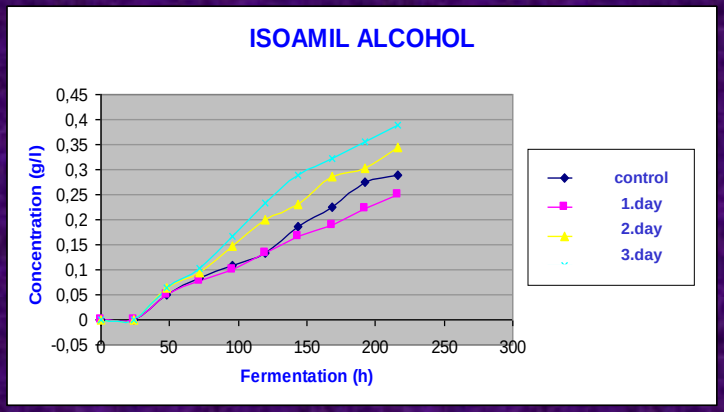


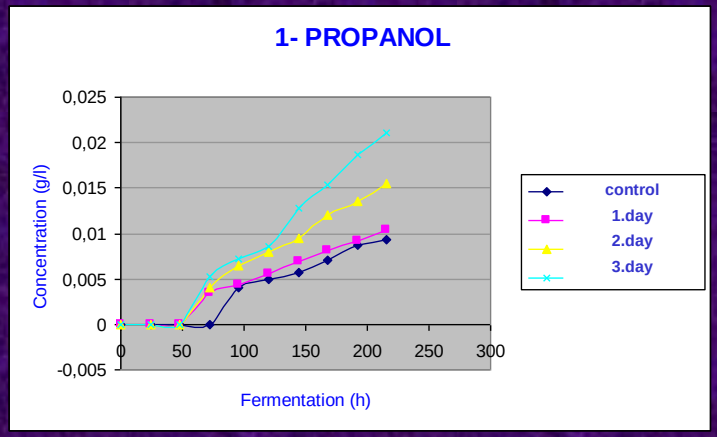


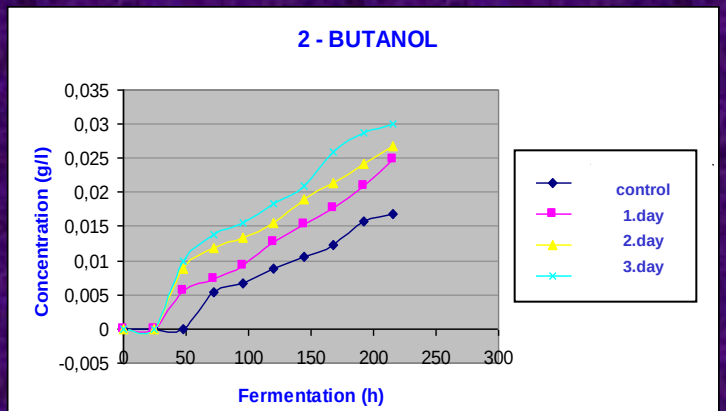


Influence of static magnetic field on *Saccharomyces cerevisiae* metabolism

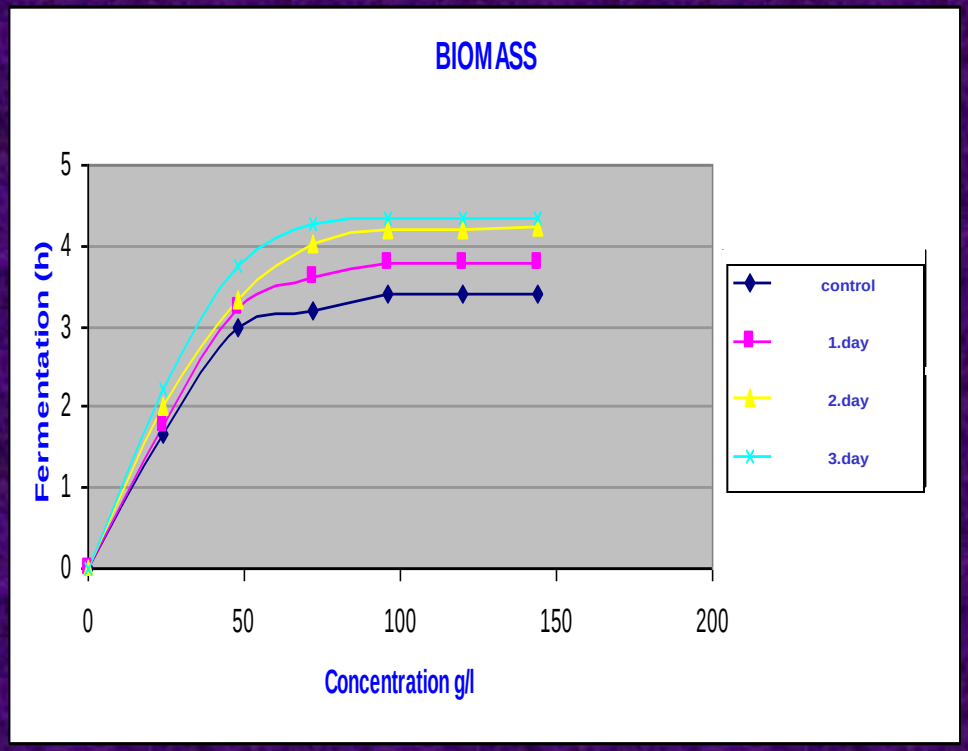


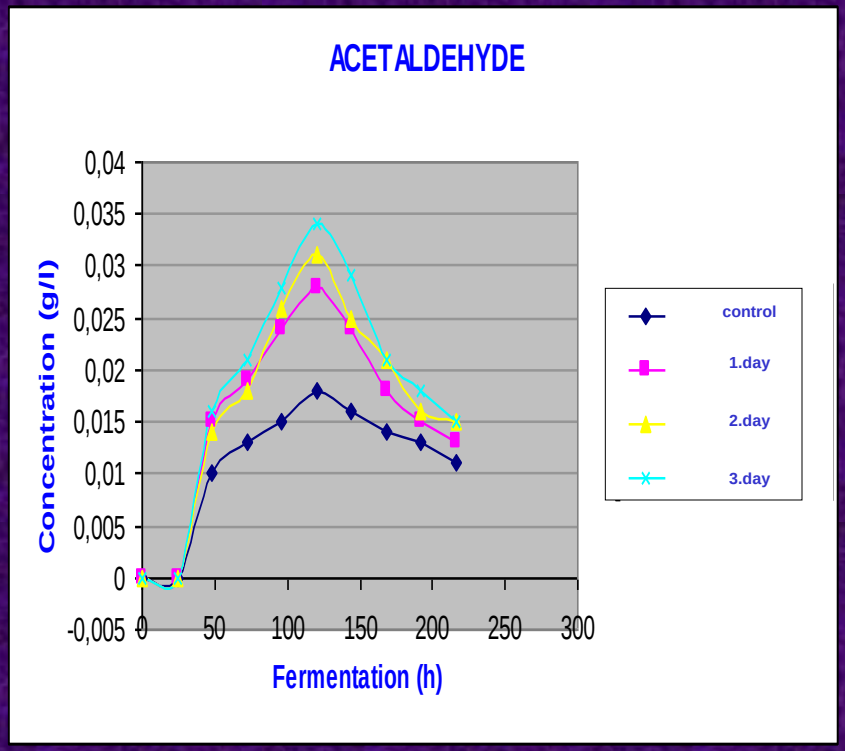




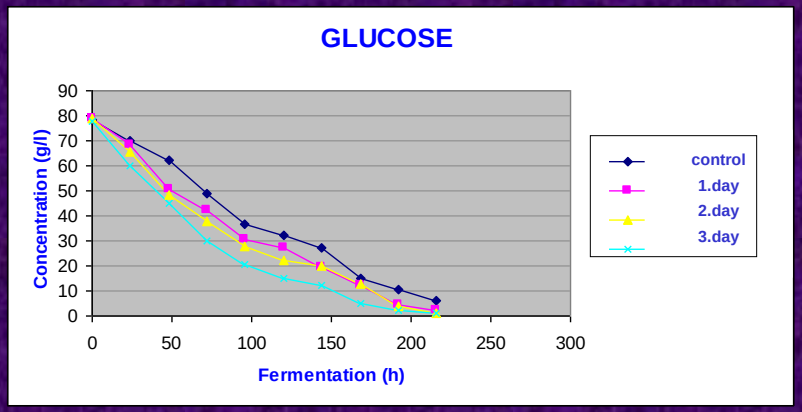


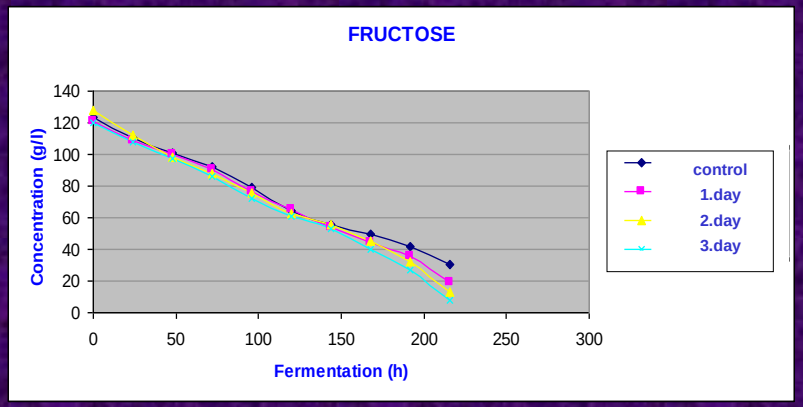
Influence of static magnetic field on *Saccharomyces cerevisiae* metabolism

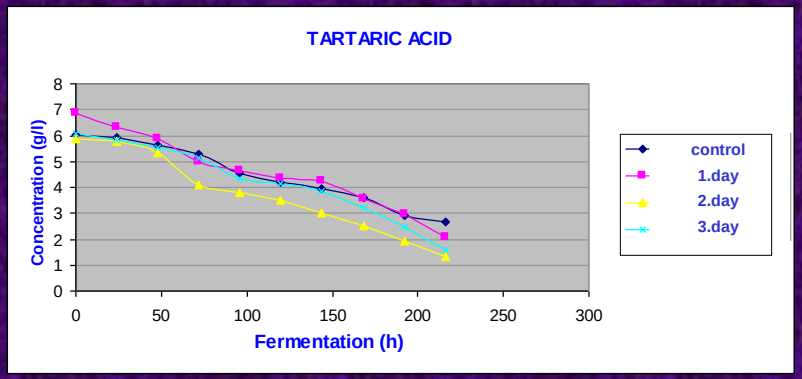


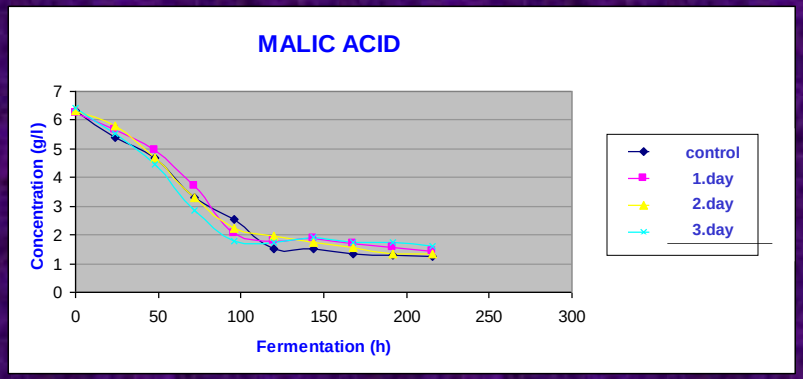


Influence of static magnetic field on *Saccharomyces cerevisiae* metabolism

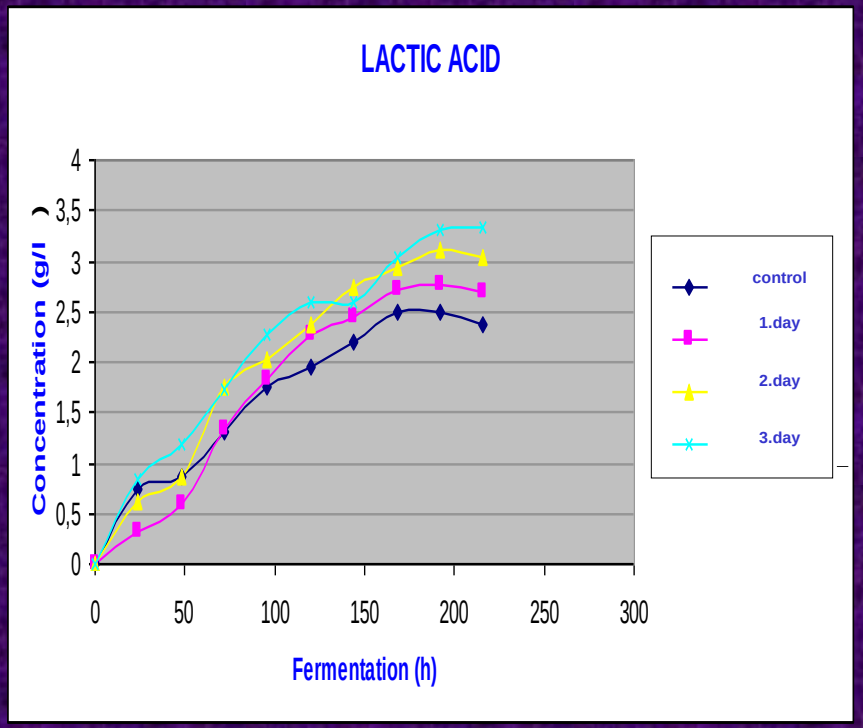


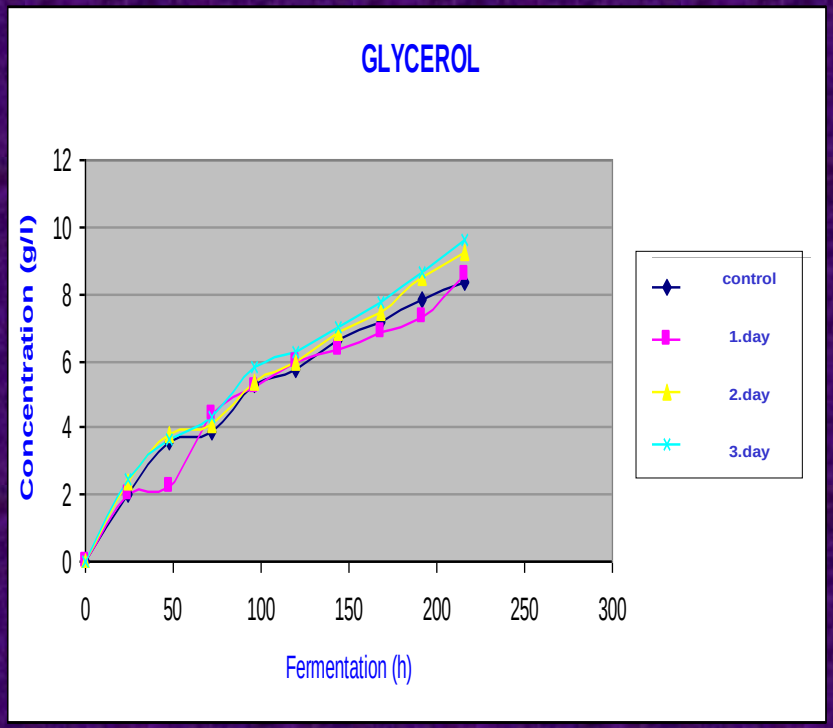




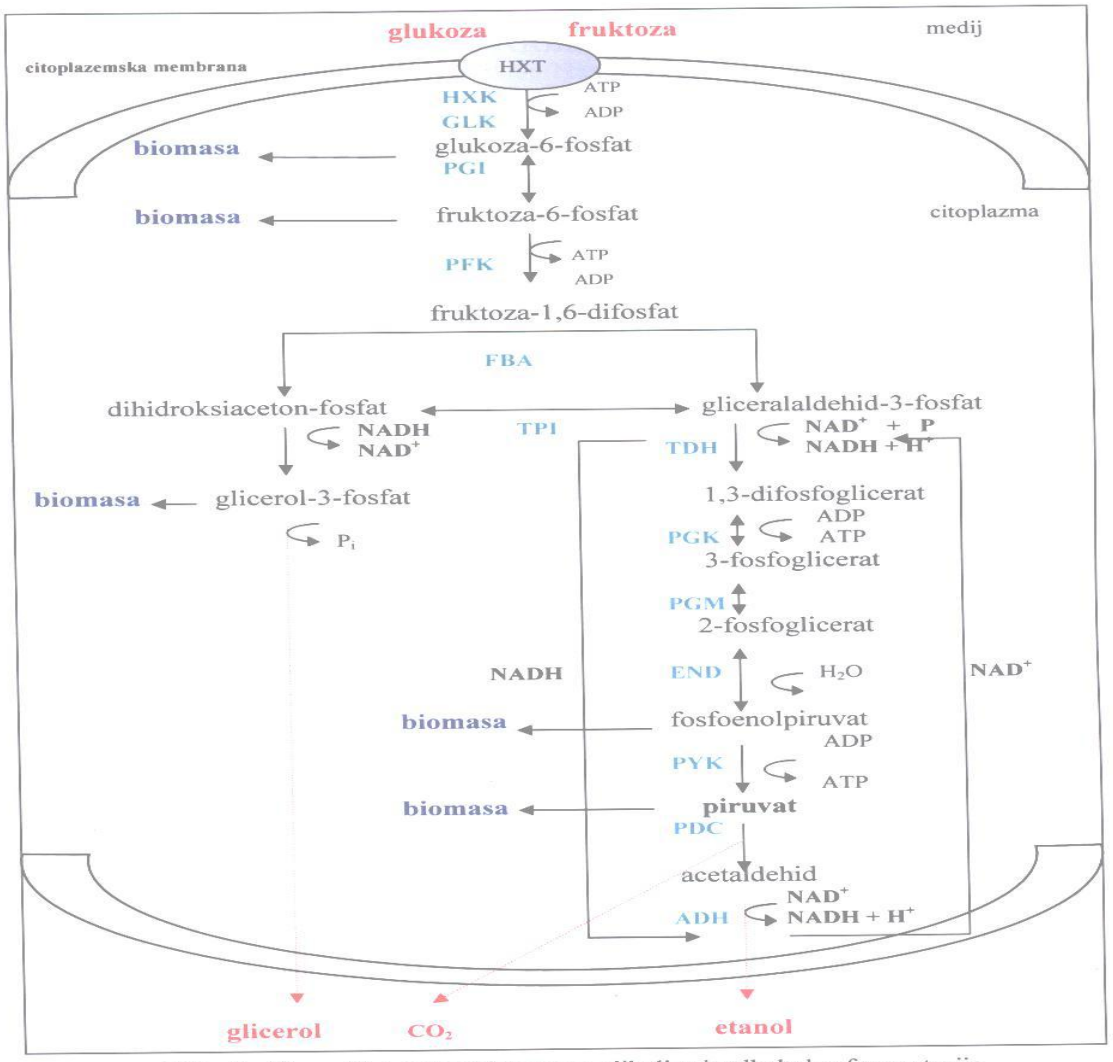


Influence of static magnetic field on *Saccharomyces cerevisiae* metabolism





Glycolisis



Slika 5.: Shematični prikaz bioprocesa glikolize in alkoholne fermentacije.

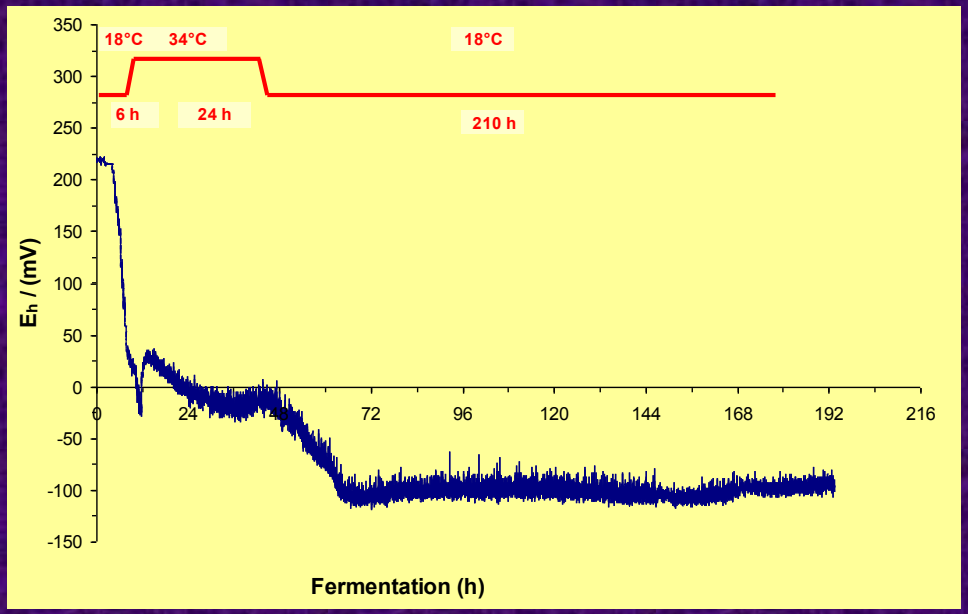
Pomen kratic na sliki: HXT (prenašalni protein za heksozo), HXK (heksokinaza), GLK (glukokinaza), PGI (fosfoglukoizomeraza), PFK (fosfofruktokinaza), FBA (aldolaza), TPI (triozafosfat izomeraza), TDH (gliceraldehid-3-fosfat dehidrogenaza), PGK (fosfoglicerat kinaza), PGM (fosfoglicerat mutaza), ENO (enolaza), PYK (piruvat kinaza), PDC (piruvat dekarboksilaza), ADH (alkohol dehidrogenaza). (Boulton *in sod.*, 1996)

FERMENTATION TEMPERATURE

- **Berovic M, Herga M. 2007 Heat shock on *Saccharomyces cerevisae* inocolum increases glycerol production in wine fermentation *Biotechnol. Lett.* vol. 29, 6, 891-894 (2007)**

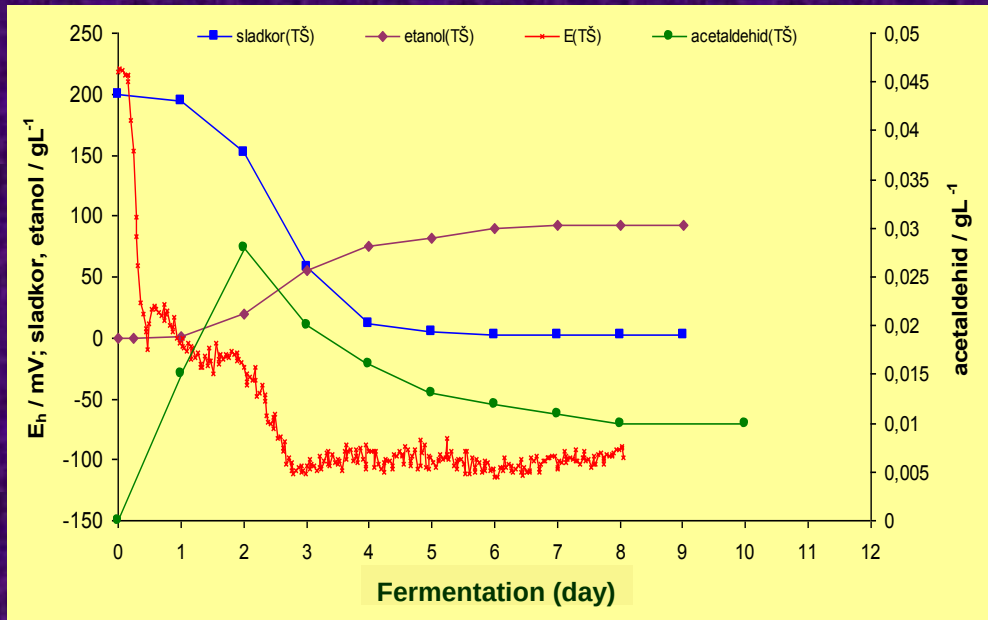
- **Berovic M, Pivec A., Kosmerl T., Wondra M., Celan S., Heat Shock on Glycerol Production in Alcohol Fermentation. *J.Biosc.Bioeng*.vol.103,2,135-139 (2007)**

Single heat shock

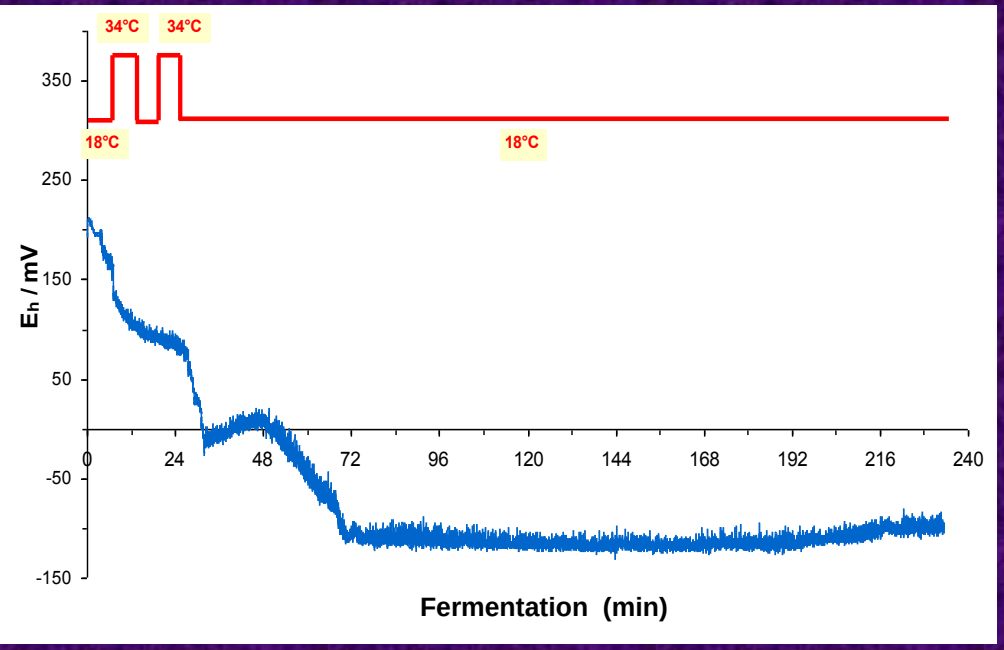


Redox potential and tempetature profile at single heat shock up to 34 °C

Redox potential, reductive sugars, acetaldehyde, ethanol, at single heat shock up to 34 °C

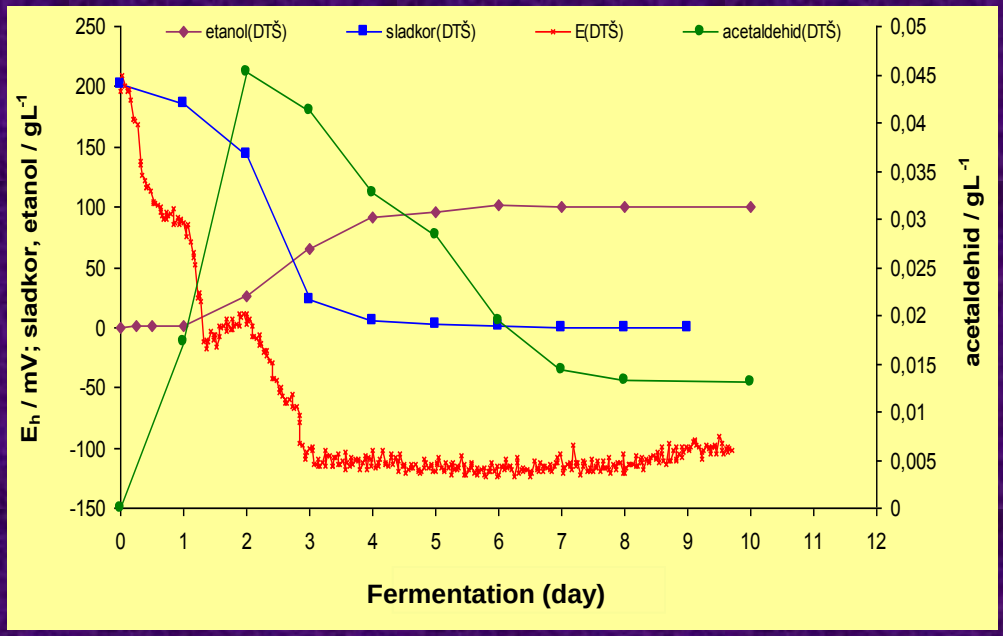


Double heat shock

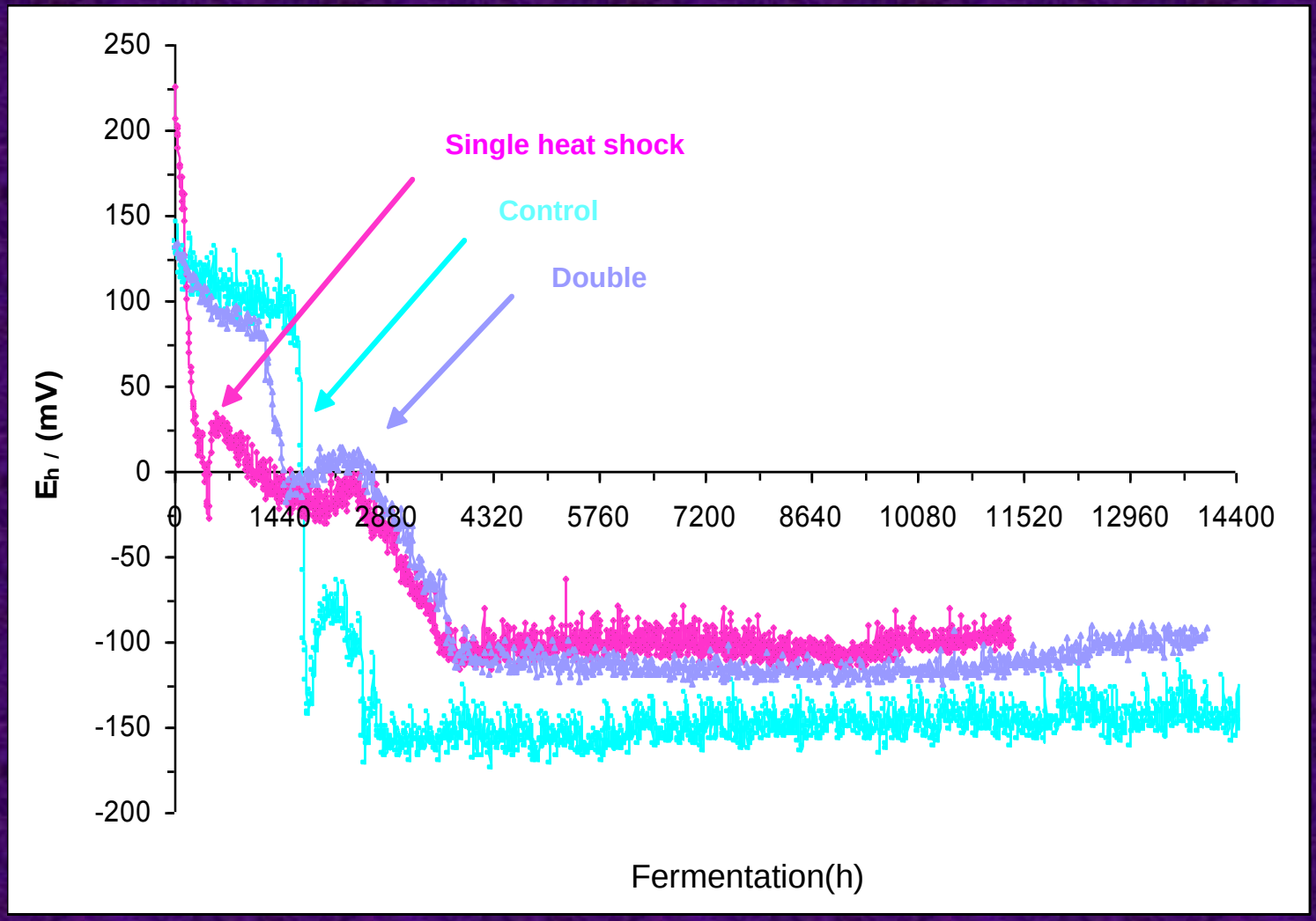


Redox potential and tempetature profile at double heat shock up to 34 °C

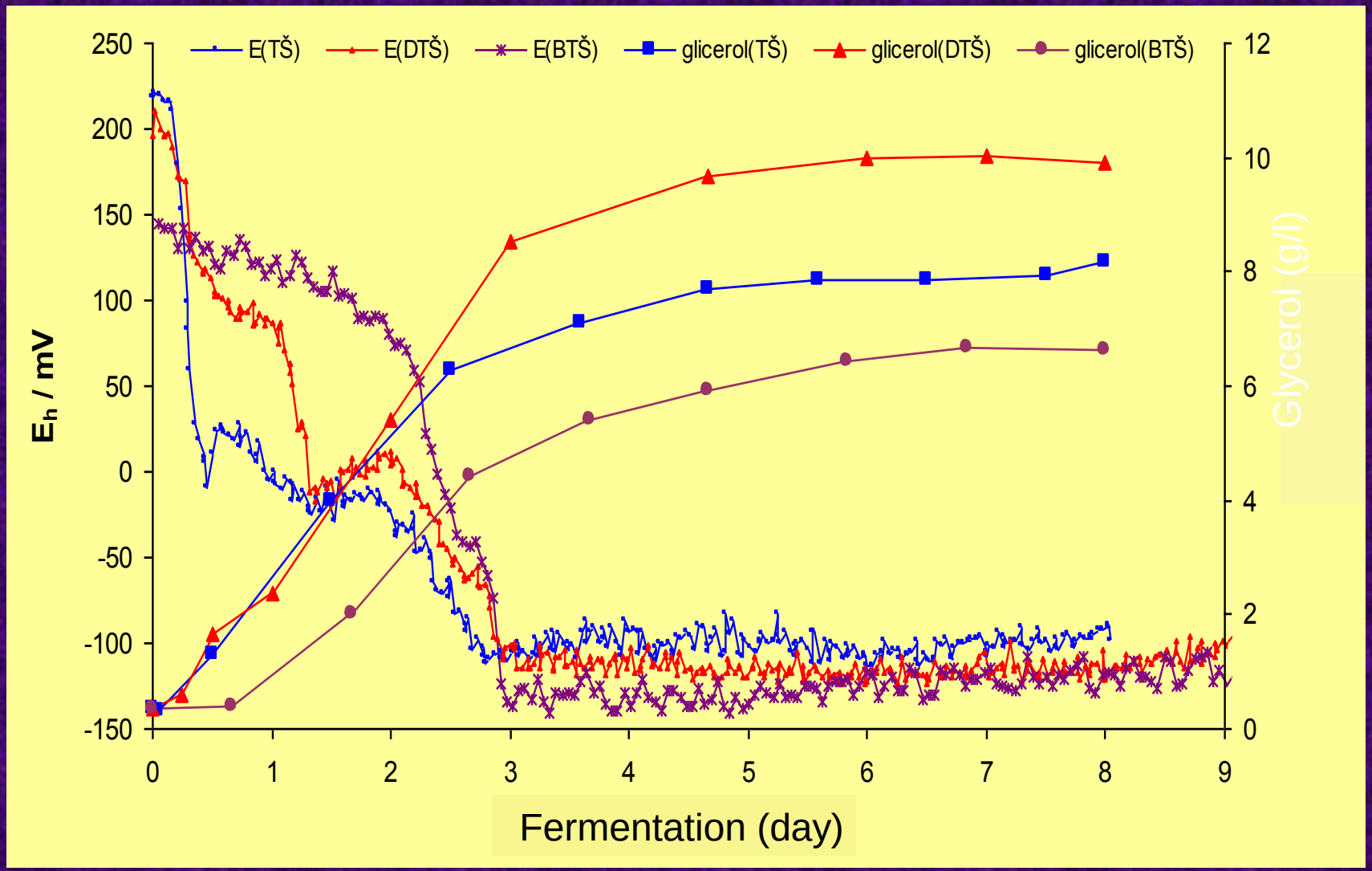
Redox potential, reductive sugars, acetaldehyde, ethanol, at double heat shock up to 34 °C



On-line redox potential at single, double heat shock and in control in *Sauvignon blanc* fermentation



Influence of heat shock on glycerol production



Influence of single and double heath shock on glicerol production

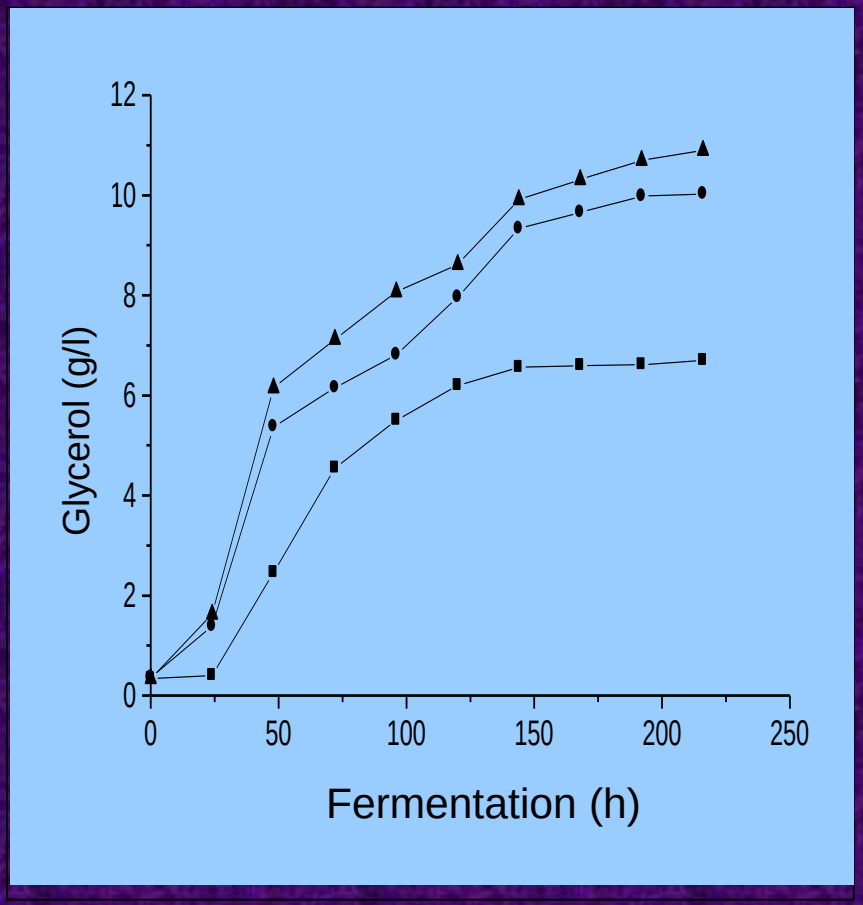


Fig. 1: Glicerol production at constant fermentation temperature of 18ºC; black square, control; black circle, single heat shock at 34ºC for 4 h; black triangle, single heat shock at 34ºC for 24 h.

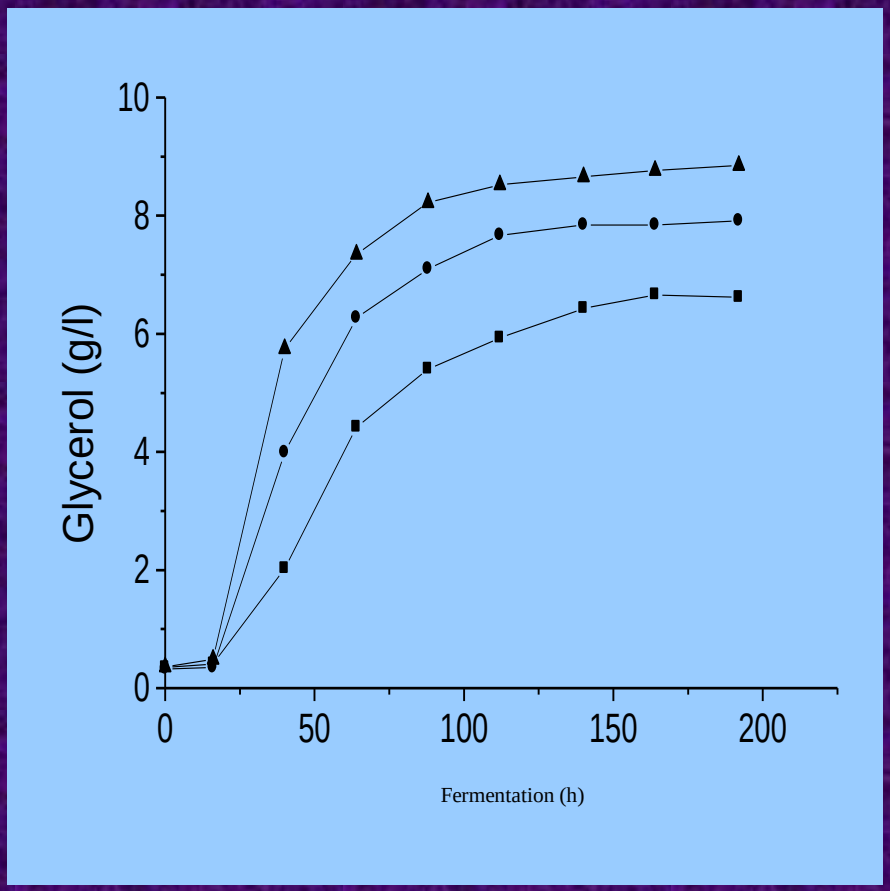
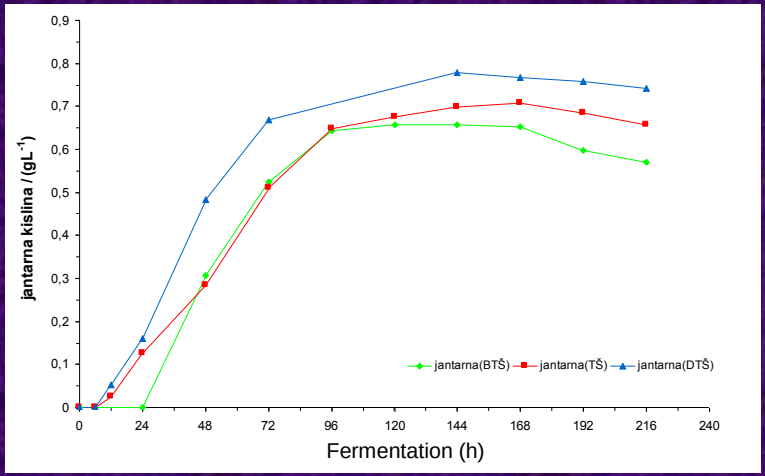
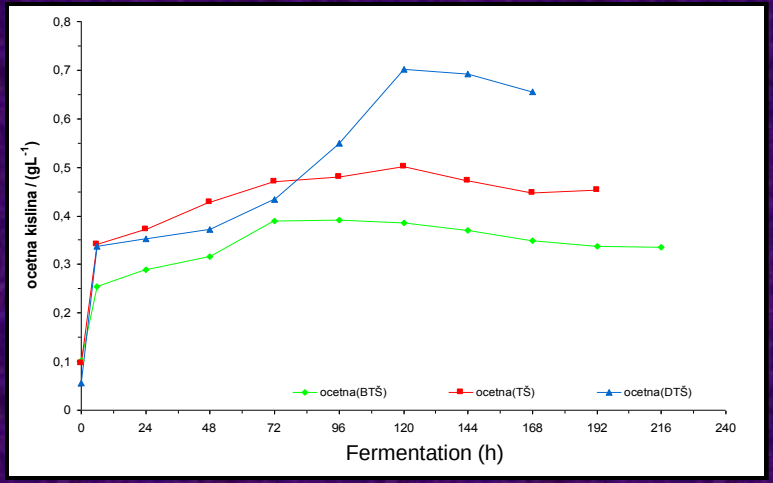


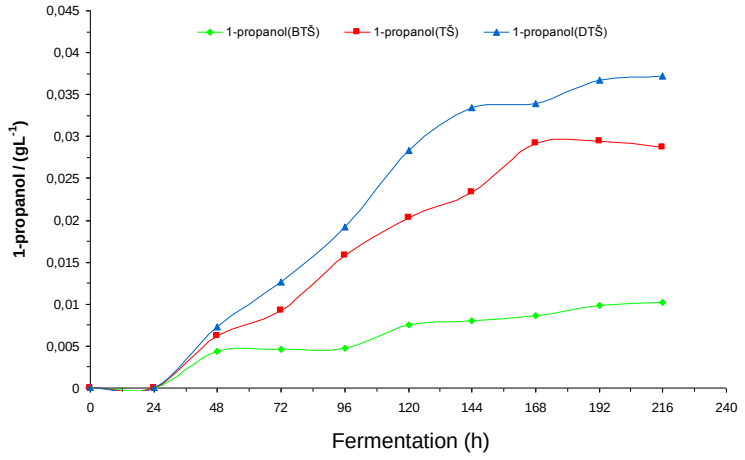
Fig. 2: Glicerol production at constant fermentation temperature of 18 ºC; black square, control; black circle, double heat shock at 34ºC (2 x 4 h); and black triangle, double heat shock at 34ºC (2 x 8 h).



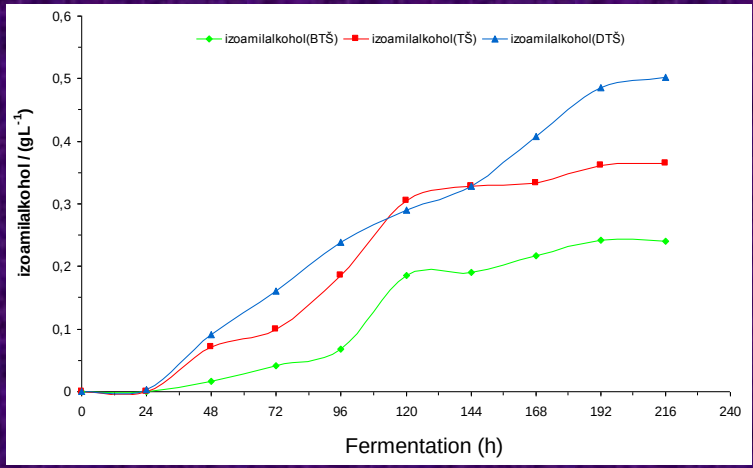
Succinic acid



Acetic acid



1-propanol



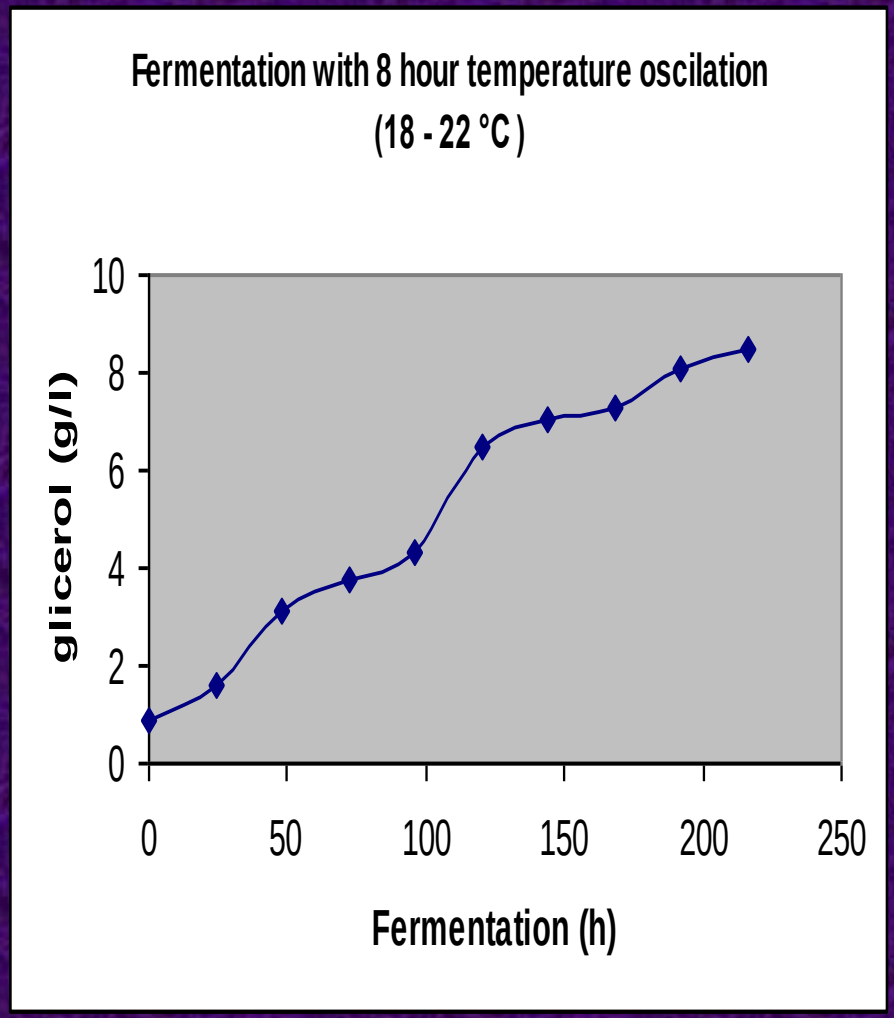
Iso-amil alcohol

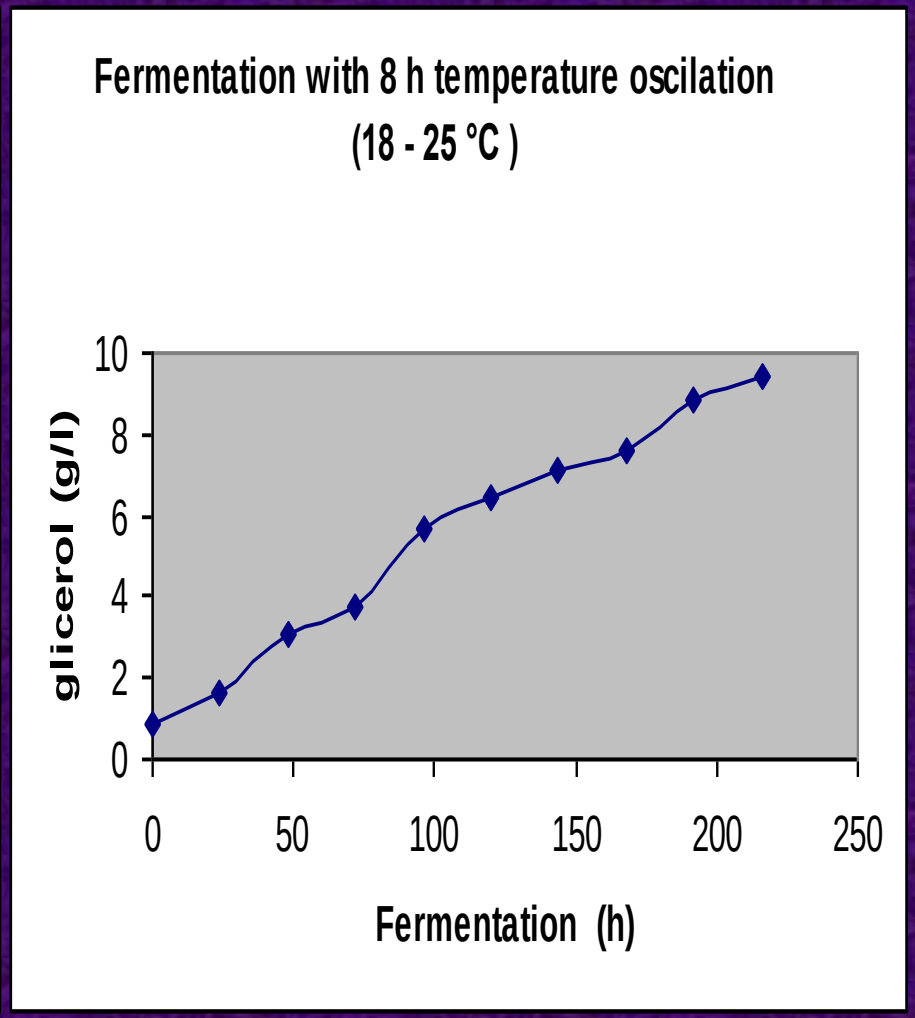
Metabolites produced in fermentations at various temperature conditions

Single HS: single heat shock for 4 h and single heat shock for 24 h
Double HS: double heat shock for 2 x 4 h and double heat shock for 2 x 8 h.

Fermentation	Reduc. sugars (g l^{-1})	Isoamyl alcohol (g l^{-1})	1-propanol (g l^{-1})	Acet aldehyde (g l^{-1})	Ethanol (g l^{-1})	E (Glycerl (g l^{-1})	Acetic acid (g l^{-1})	Succ. acid (g l^{-1})	Citric acid (g l^{-1})	Malic acid (g l^{-1})	Tartaric acid (g l^{-1})
Control (18°C)	3.2	0.24	0.090	0.009	86.90	6.7	0.35	0.67	0.58	2.9	2.5
Single HS (4h)	2.6	0.33	0.031	0.010	92.00	7.9	0.45	0.73	0.57	2.5	3.5
Single HS (24h)	2.3	0.35	0.033	0.012	94.00	8.2	0.41	0.74	0.58	2.5	3.5
Double HS (2x4h)	2.0	0.51	0.037	0.013	101.00	10.0	0.56	0.78	0.72	2.3	3.6
Double HS (2x8h)	1.9	0.53	0.039	0.014	103.11	10.9	0.56	0.79	0.72	2.1	3.5

Biosynthesis of glycerol at temperature oscilation





Influence of heat shock on *Sacharomyces cerevisiae* inoculum

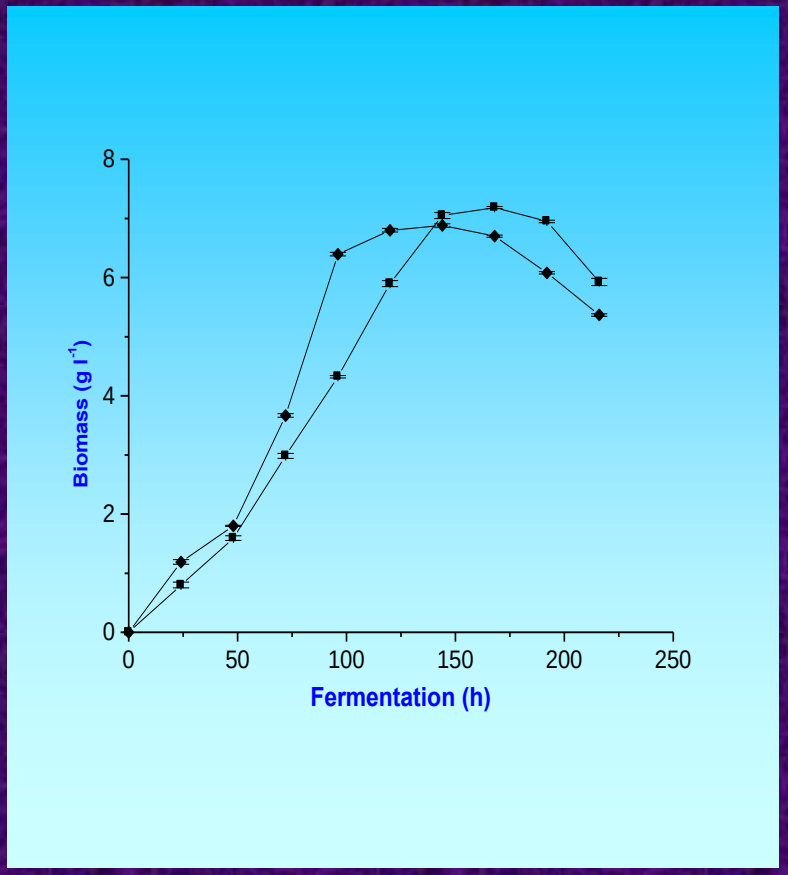


Fig.1. Production of biomass with inoculum ■ exposed for 20 min to the heat shock at 45 °C and ♦ control at 18 ºC.

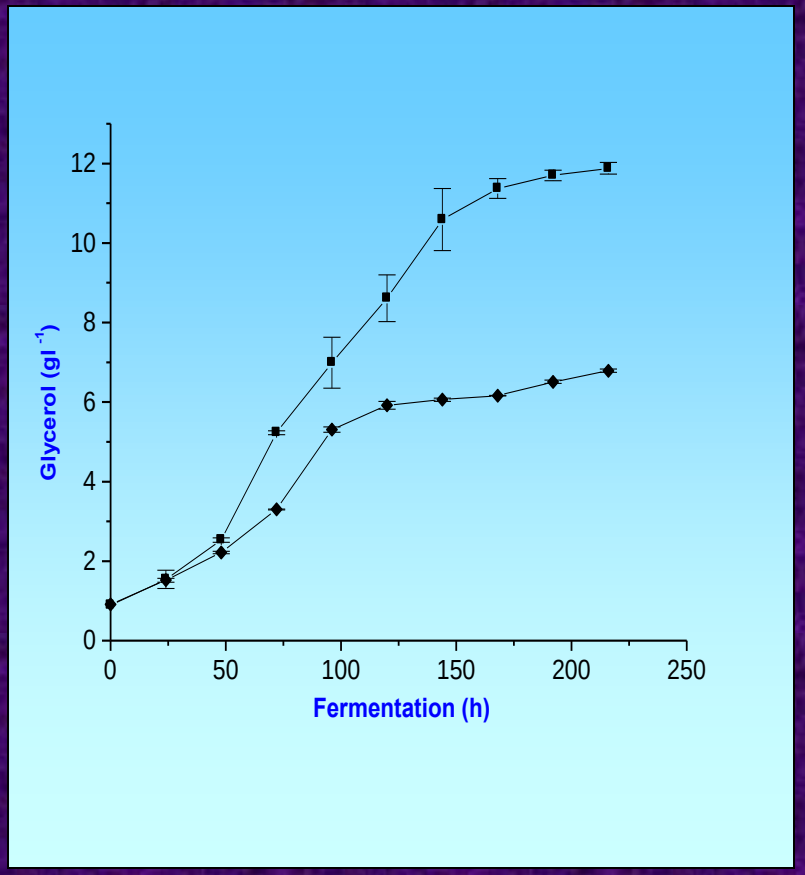
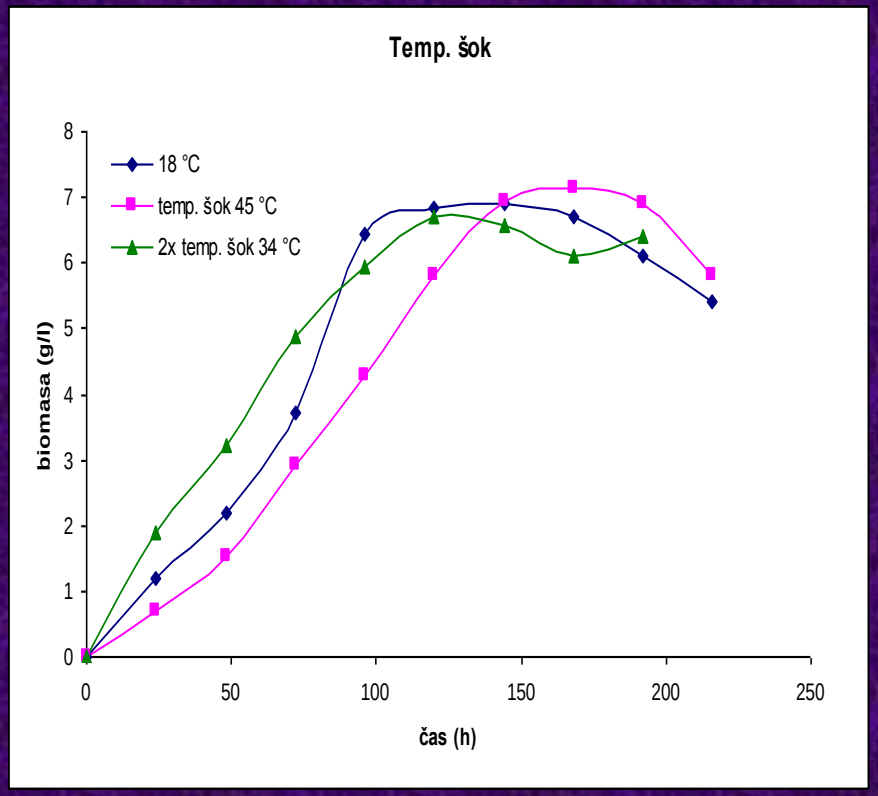
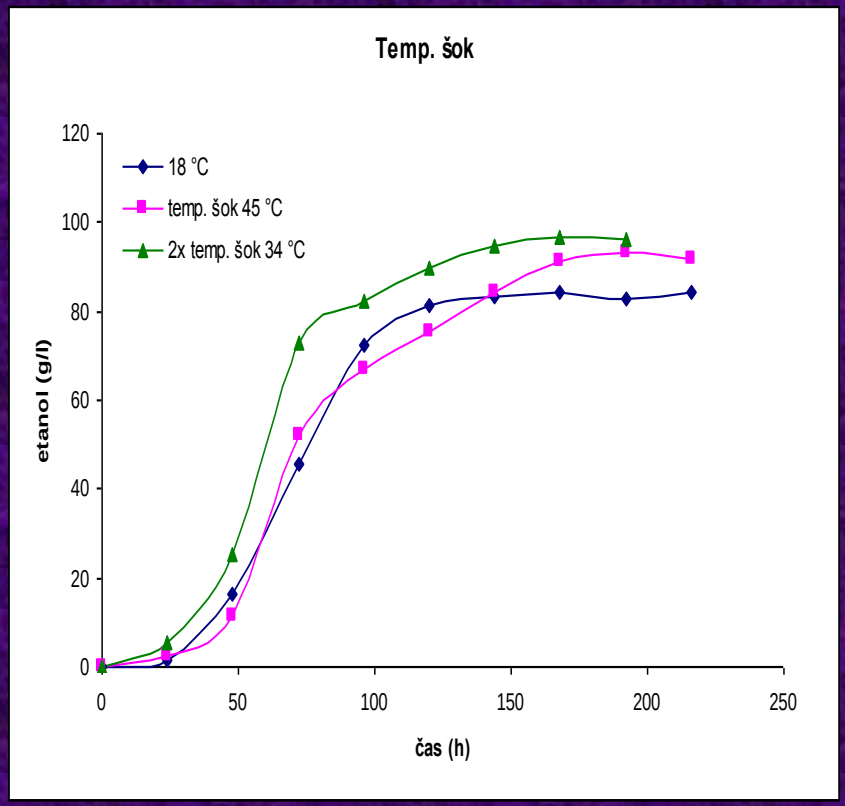
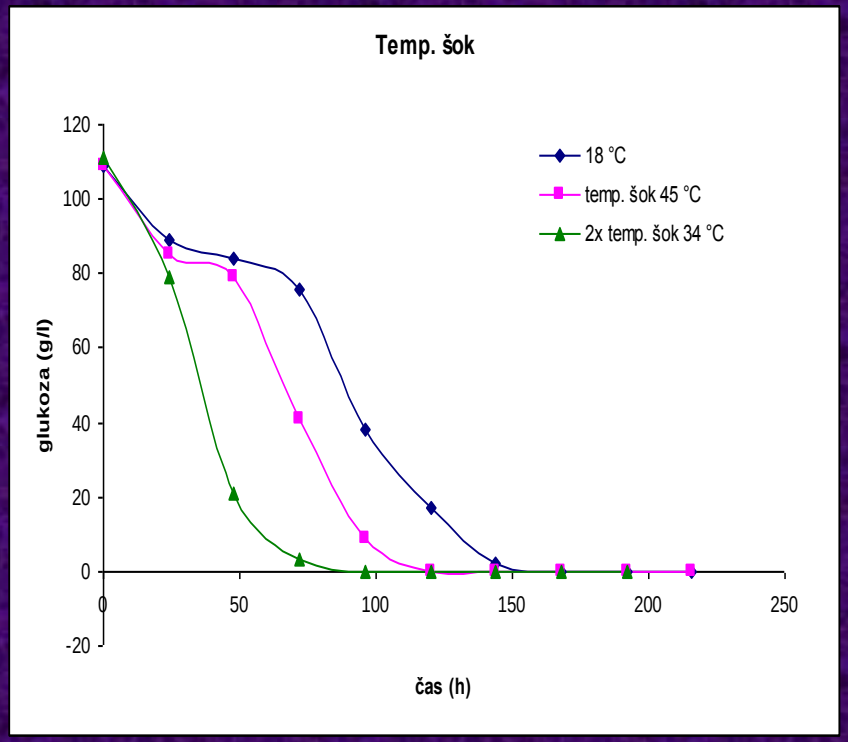


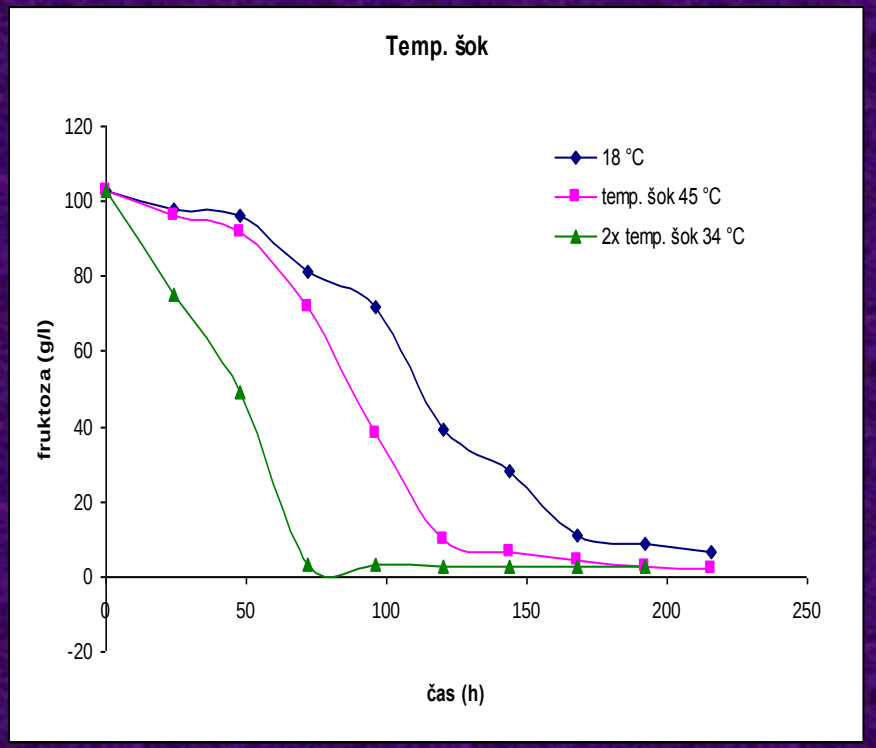
Fig.2 Glycerol biosynthesis ■ with inoculum exposed for 20 min to the heat shock at 45 °C and ♦ control at 18 ºC.



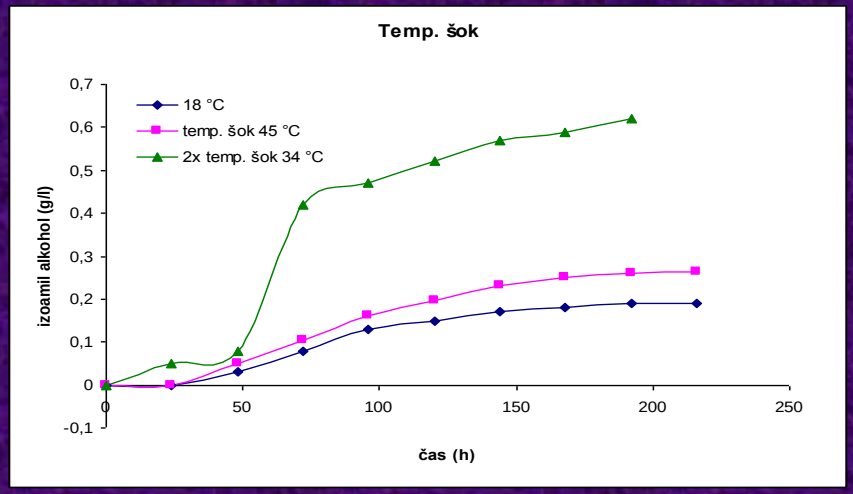


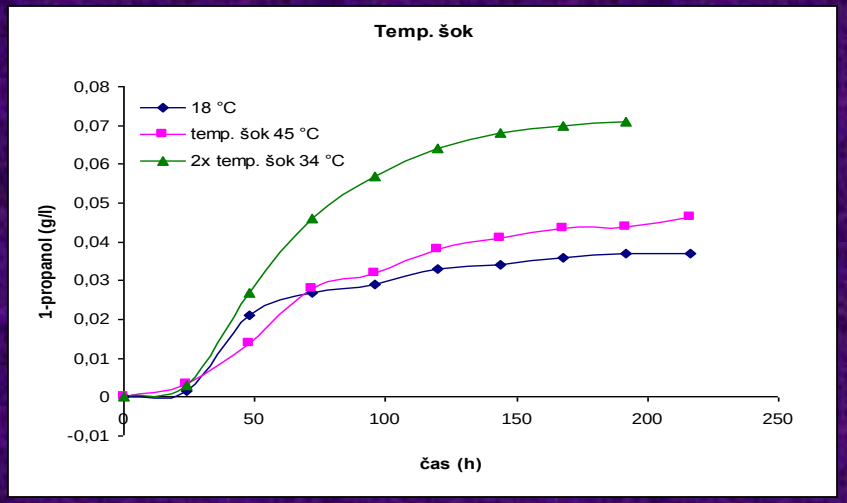
Naraščanje biomase in etanola med fermentacijo pri 18°C, 20 minutnem temperaturnem šoku na vcepku pri 45°C in dvakratnem 12 urnem temperaturnem šoku pri 34 °C

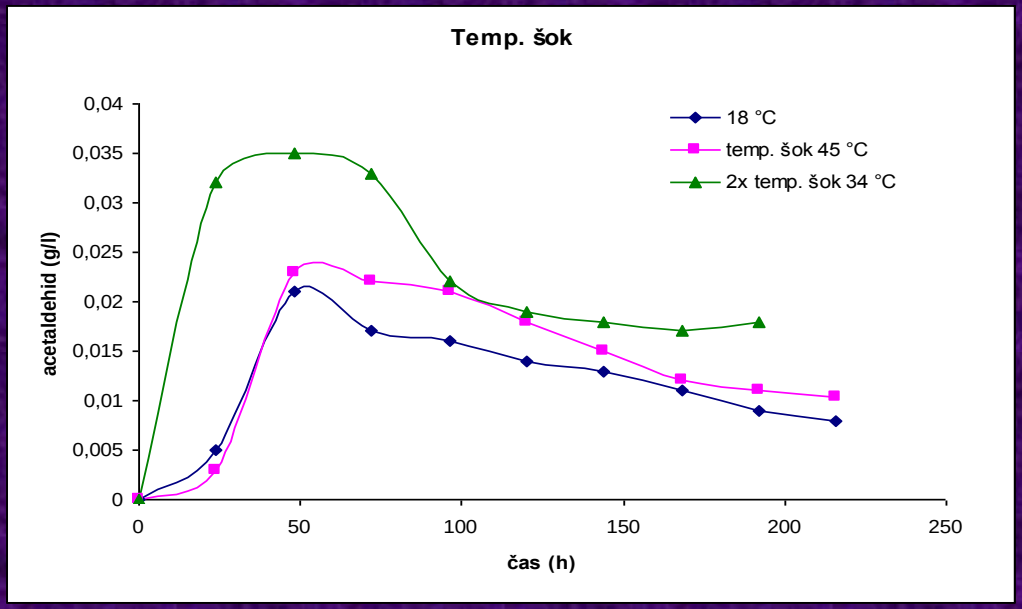




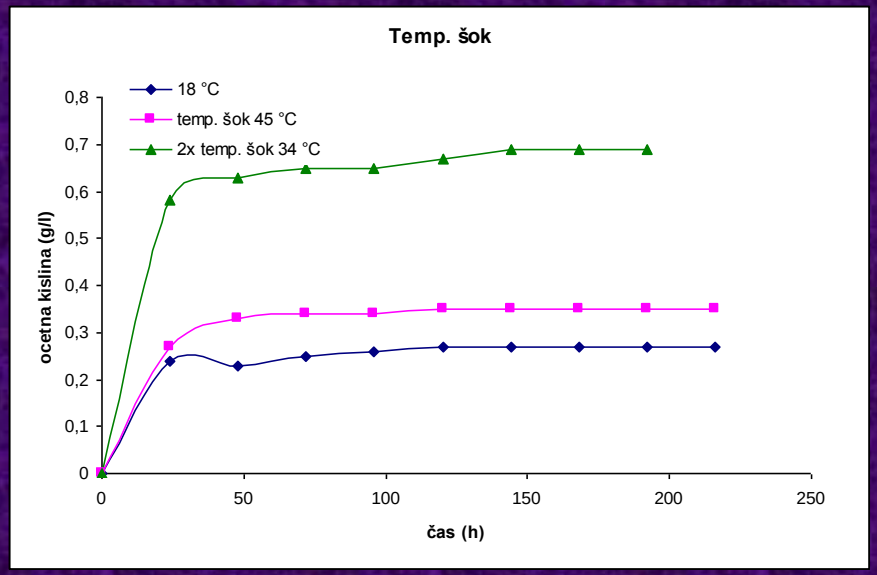
Poraba glukoze in fruktoze med fermentacijo pri 18°C, 20 minutnem temperaturnem šoku na vcepku pri 45°C in dvakratnem 12 urnem temperaturnem šoku pri 34 °C

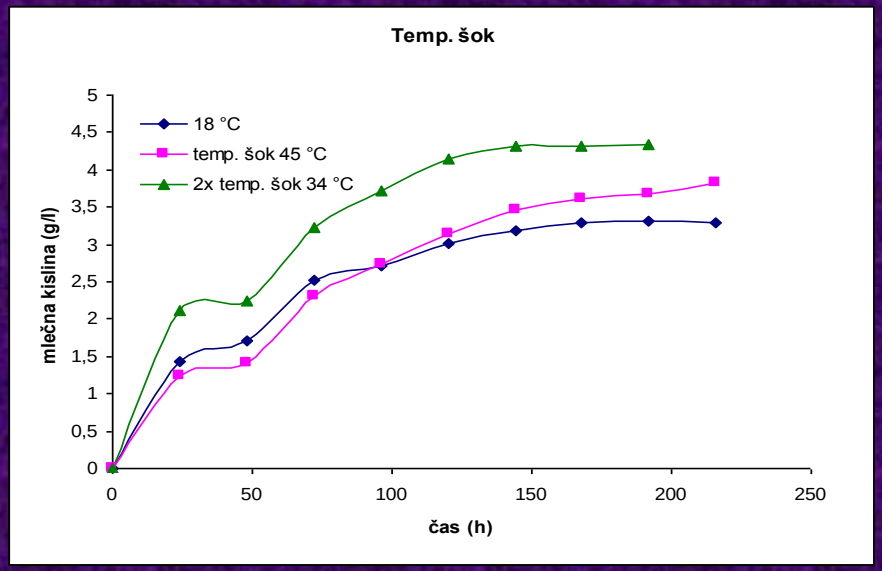


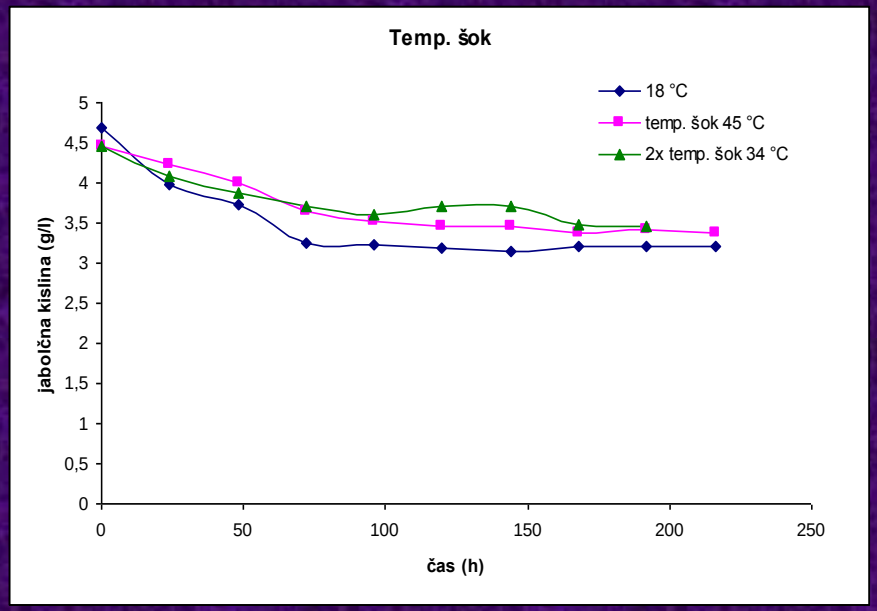


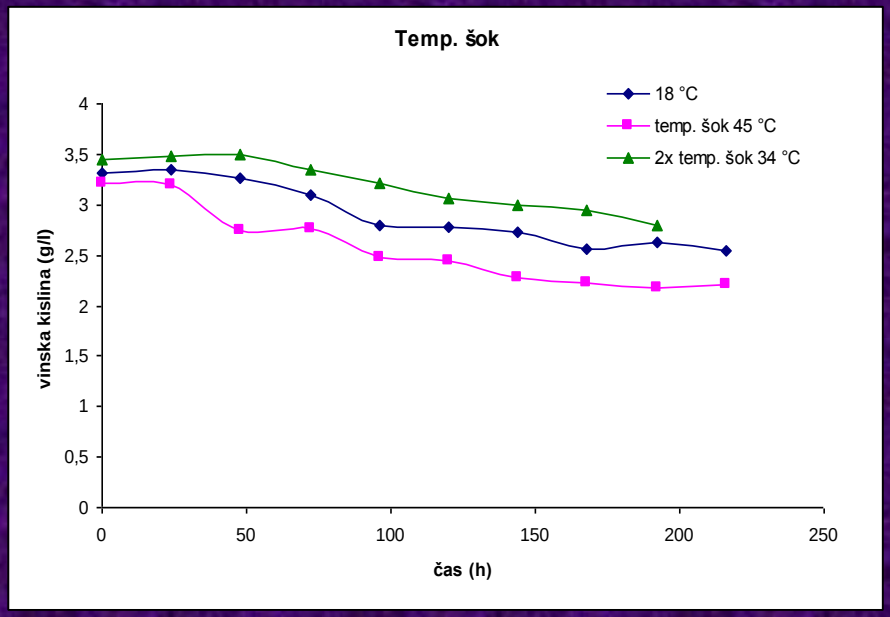


Nastajanje izoamilalkohola, 1-propanola in acetaldehida med fermentacijo pri 18°C, 20 minutnem temperaturnem šoku na vcepku pri 45°C in dvakratnem 12 urnem temperaturnem šoku pri 34 °C









Nastajanje ocetne in mlečne kisline, upadanje vinske in jabolčne kisline med fermentacijo pri 18°C, 20 minutnem temperaturnem šoku na vcepku pri 45°C in dvakratnem 12 urnem temperaturnem šoku pri 34 °C

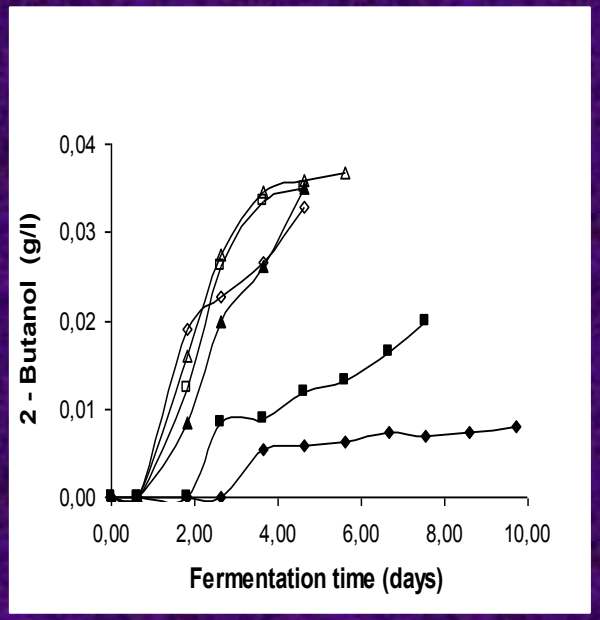
Production of glycerol with various methods

Ferm.	**Cont.** 18ºC	**Single shock** 8h/34ºC	**Oscilations 12h** 18-25ºC	**Inoc. shock** 20min/45ºC
Glycerol (g/l)	**6,81**	**9,42**	**11,62**	**12,14**

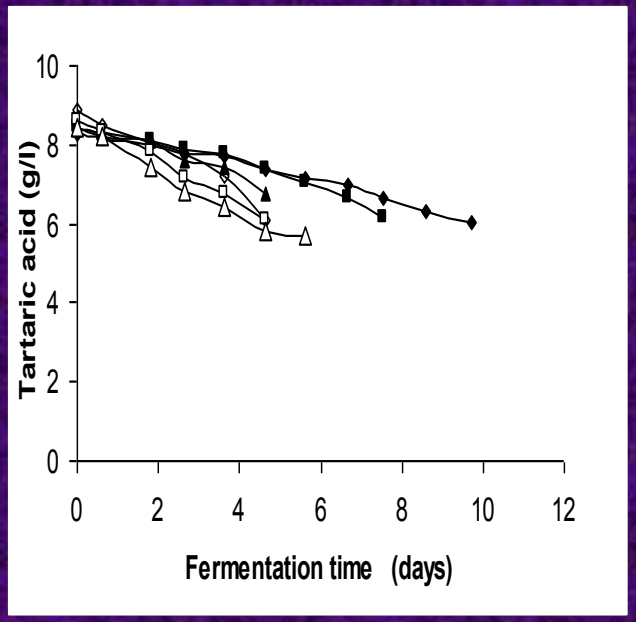
Table 1. Biomass and metabolites of control fermentation and heat shocked inoculum.

Products	Control (18 °C) (g l^{-1})	Heath shocked inoculum (20 min / 45 °C) (g l^{-1})
Biomass	6.7 ± 0.02	7.2 ± 0.02
Red. sugars	6.5 ± 0.23	2.4 ± 0.09
Acetic acid	0.28 ± 0.06	0.32 ± 0.02
Succinic acid	0.63 ± 0.11	0.75 ± 0.12
Malic acid	3.3 ± 0.16	3.4 ± 0.17
Tartaric acid	2.5 ± 0.18	2.2 ± 0.09
Ethanol	84.70 ± 2.18	91.77 ± 0.93
Glycerol	6.81 ± 0,06	12.14 ± 0,26

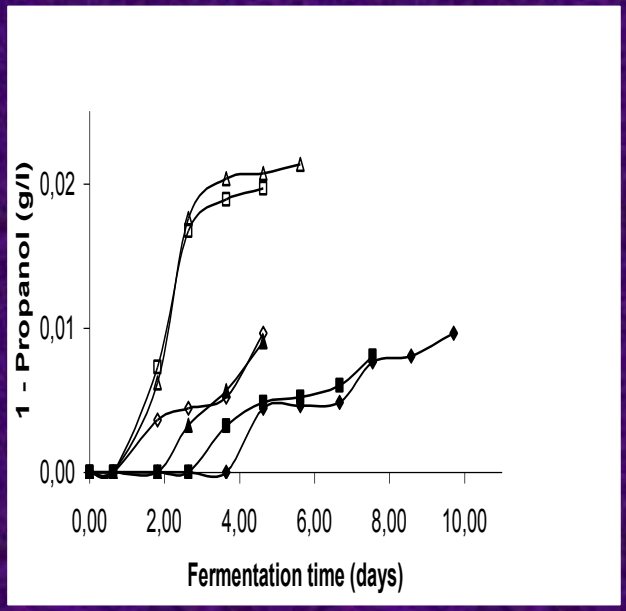
The Influence of Galvanic Field on *Saccharomyces cerevisiae* in Grape Must Fermentation



2-Butanol



Tartaric acid



1-Propanol

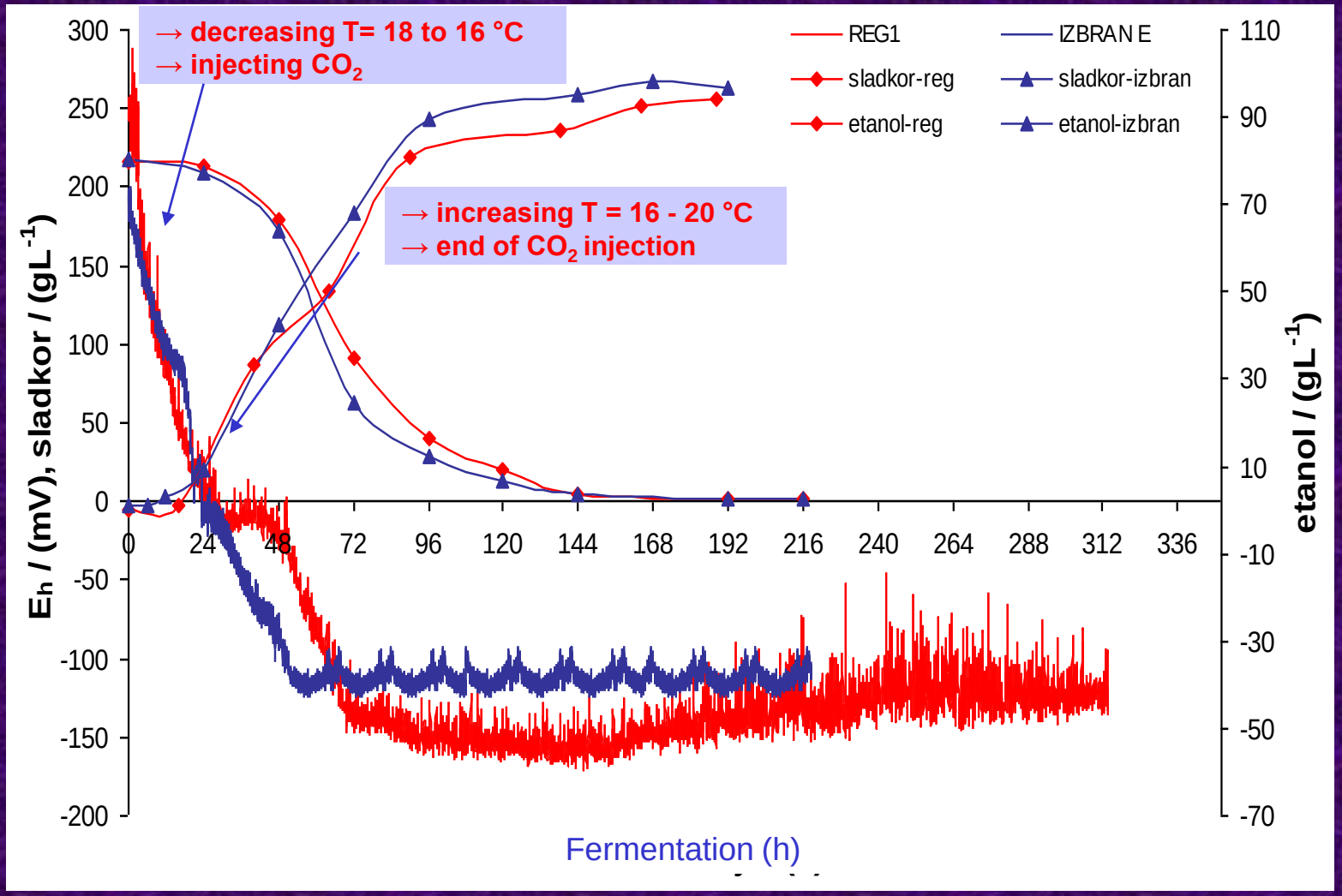
WINE FERMENTATION PROCESS CONTROL

Redox control process regulation

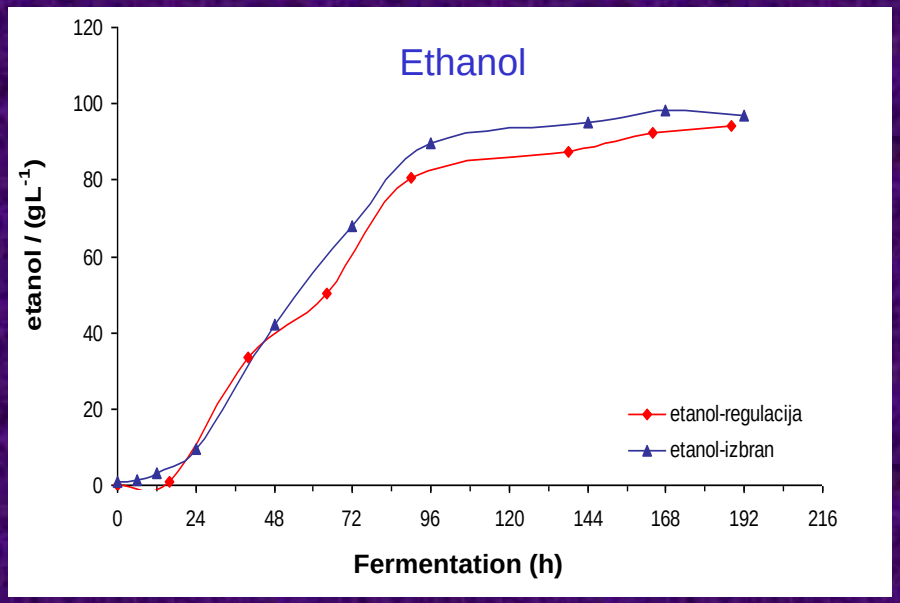
Temperature regulation

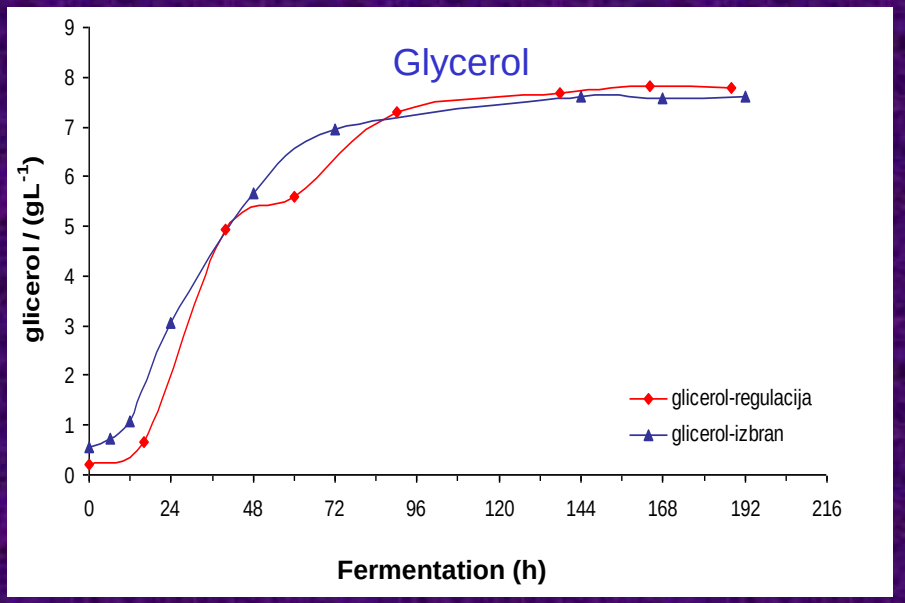
CO_2 pulses

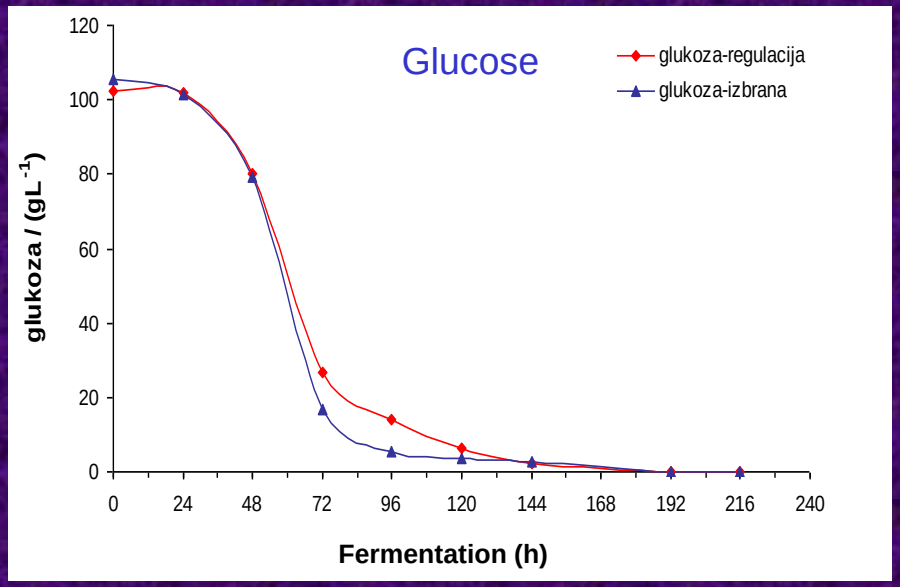
Izbran profil redoks potenciala in poskus regulacije

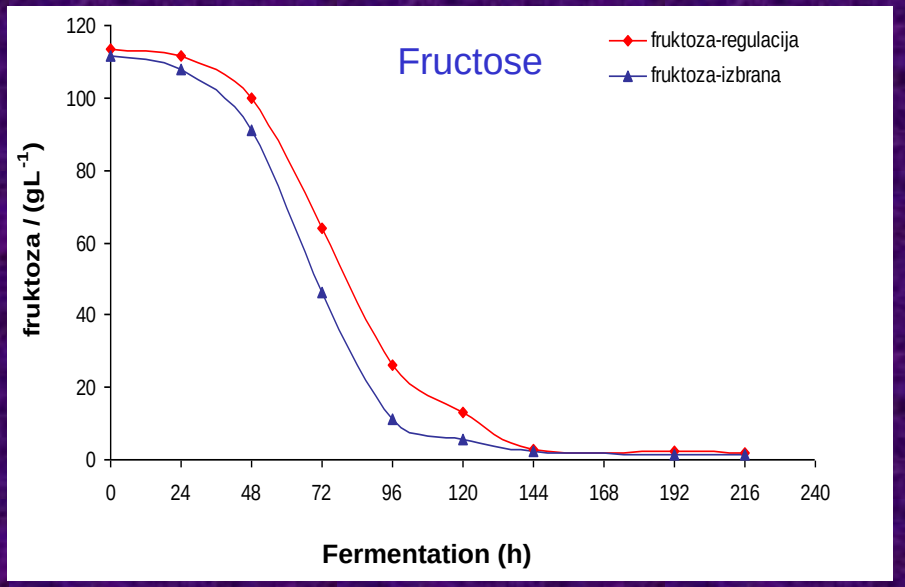


Glucose, fructose comsumtion and ethanol and glycerol production at redox controlled process

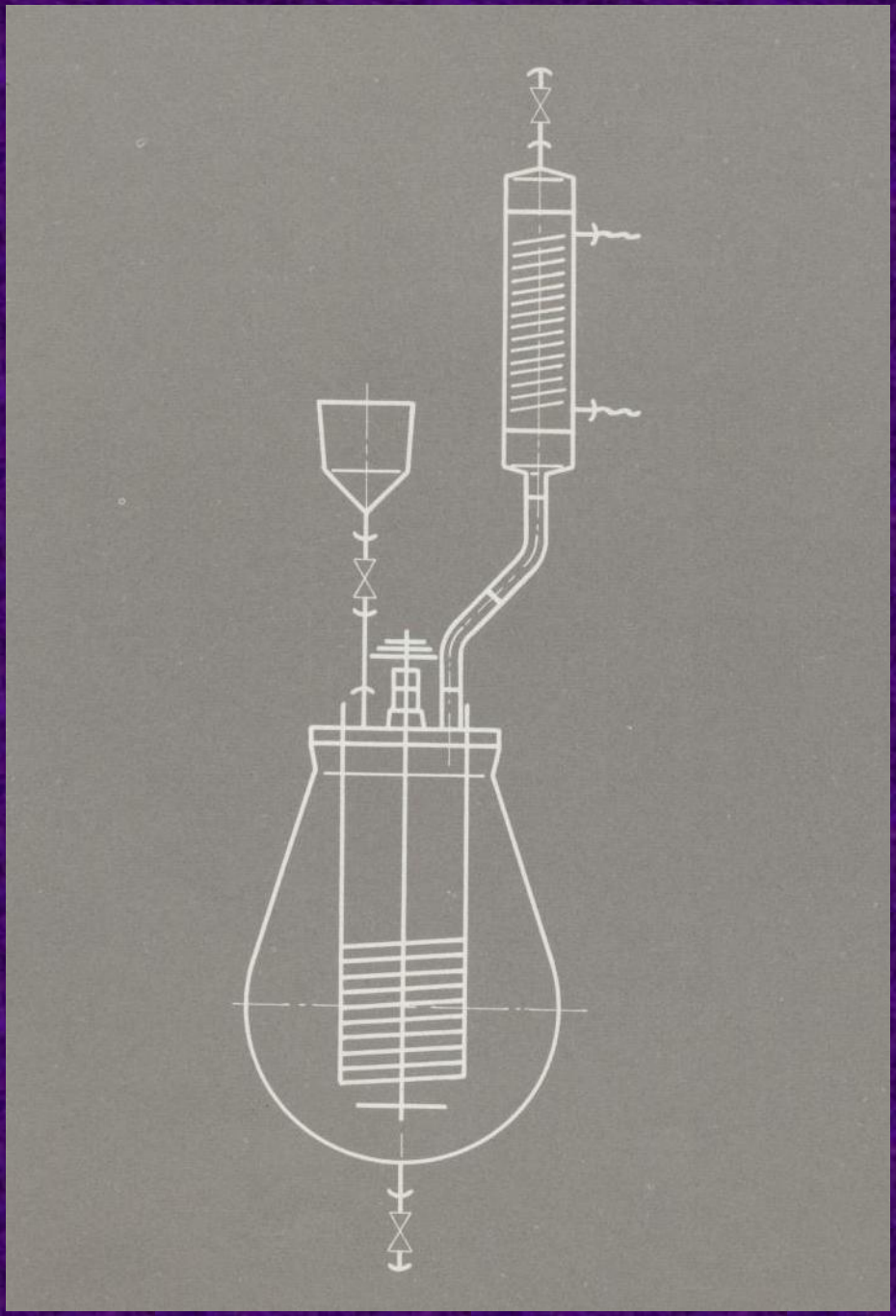




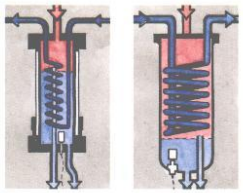




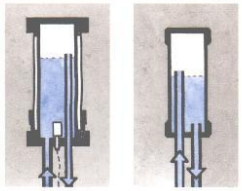
OTHER METHODS



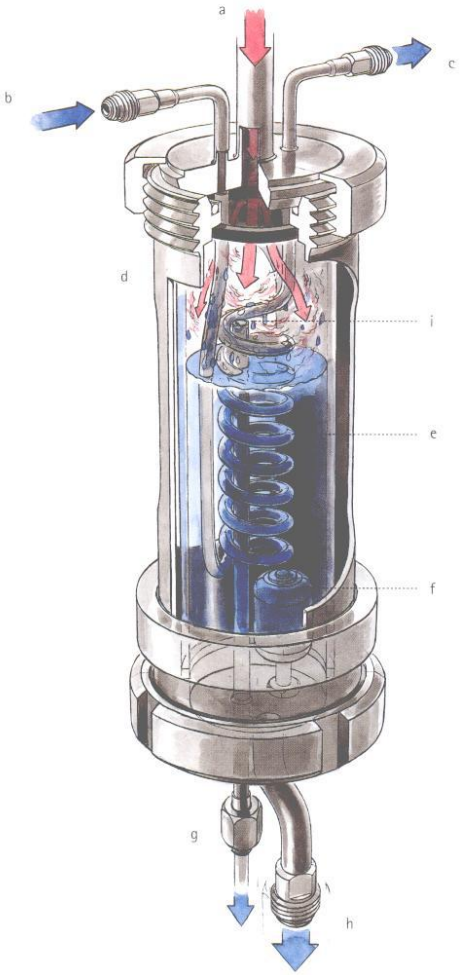
REFLUX AROMA CONDENSERS



Pressurized condensate vessel.
Left: sight glass design, right: steel vessel. Both with float switch.



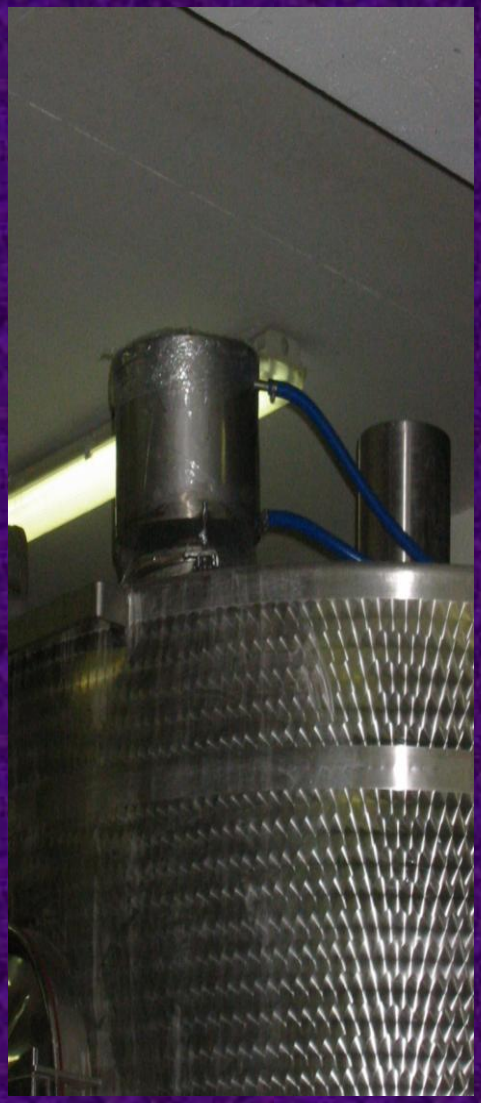
Non-pressurized balancing vessel, with and without float switch. Manual refilling.



REFLUX AROMA CONDENSERS

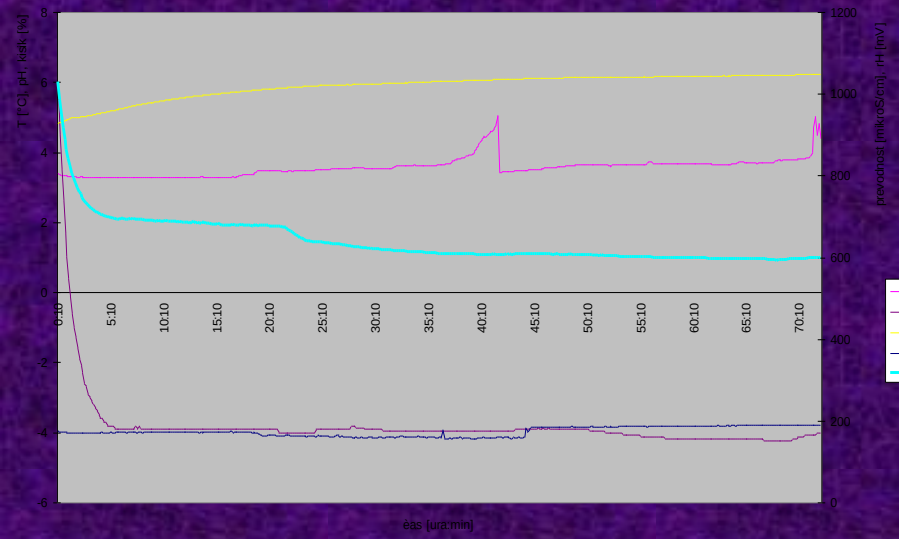
LARGE SCALE REFLUX AROMA CONDENSERS

REFLUX AROMA CONDENSERS

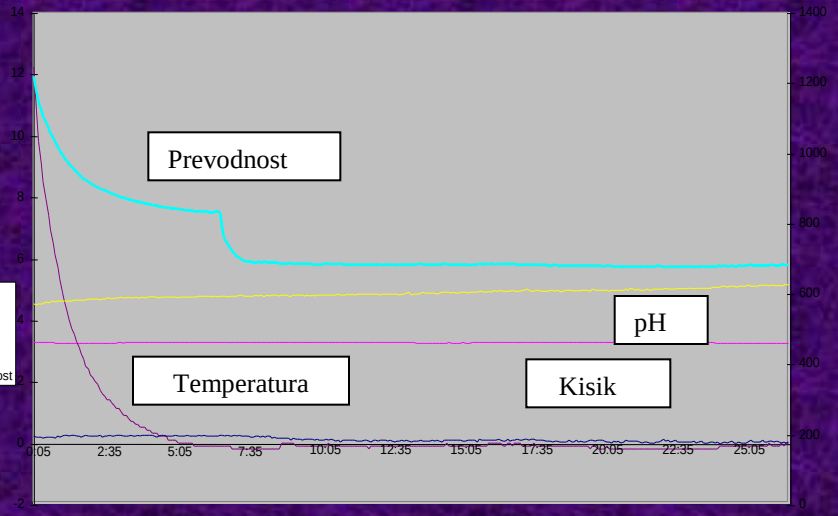


Crystalisation of potassium hidrogen tartarate in *Sauvignon blanc* wine

at 0°C with precipitation of KTH at *on-line* conductivity process control



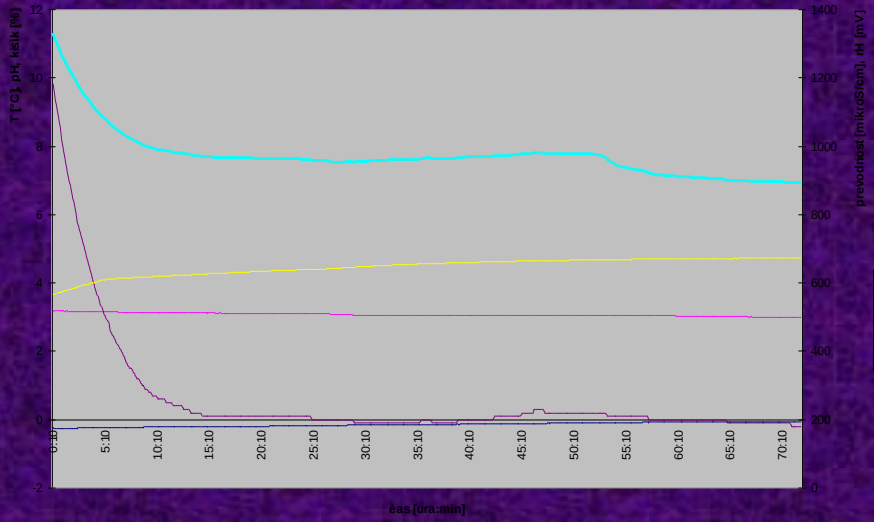
Cooling T=0 °C, termostation time 72 h



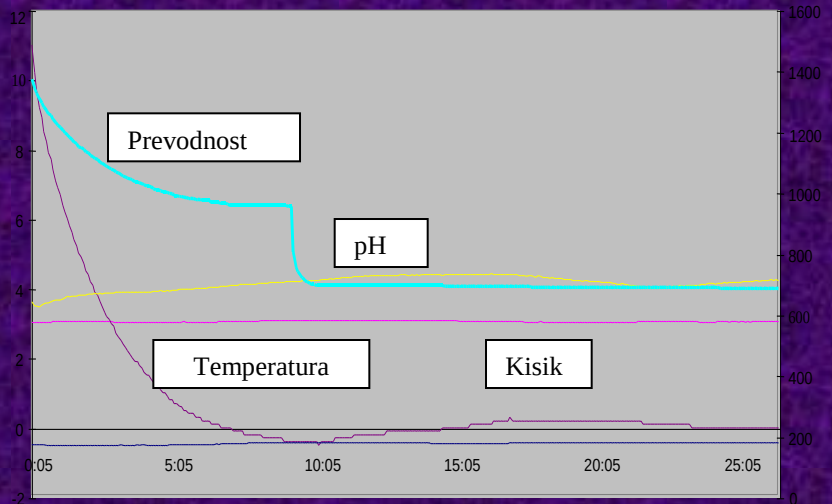
Cooling on 0°C with addition of 6g/l KHT conductivity *on-line* process control

Crystalisation of potassium hidrogen tartarate in *Malvasia* wine

at 0°C with precipitation of KTH at *on-line* conductivity process control



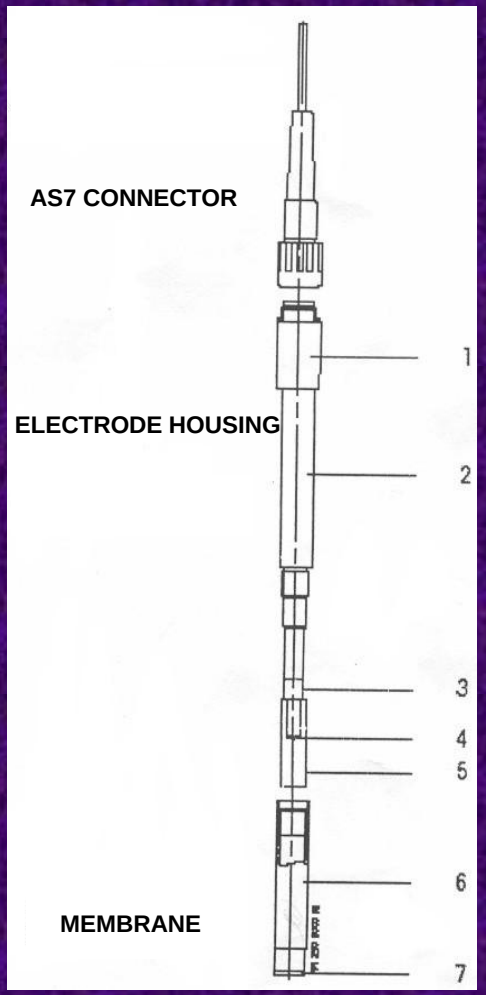
Cooling T=0 °C, termostation time 72 ur



Cooling on 0°C with addition of 6g/l KHT conductivity *on-line* process control

Sensor for SO_2 measurement

on-line measurement of free SO_2
with
modifyed CO_2 electrode

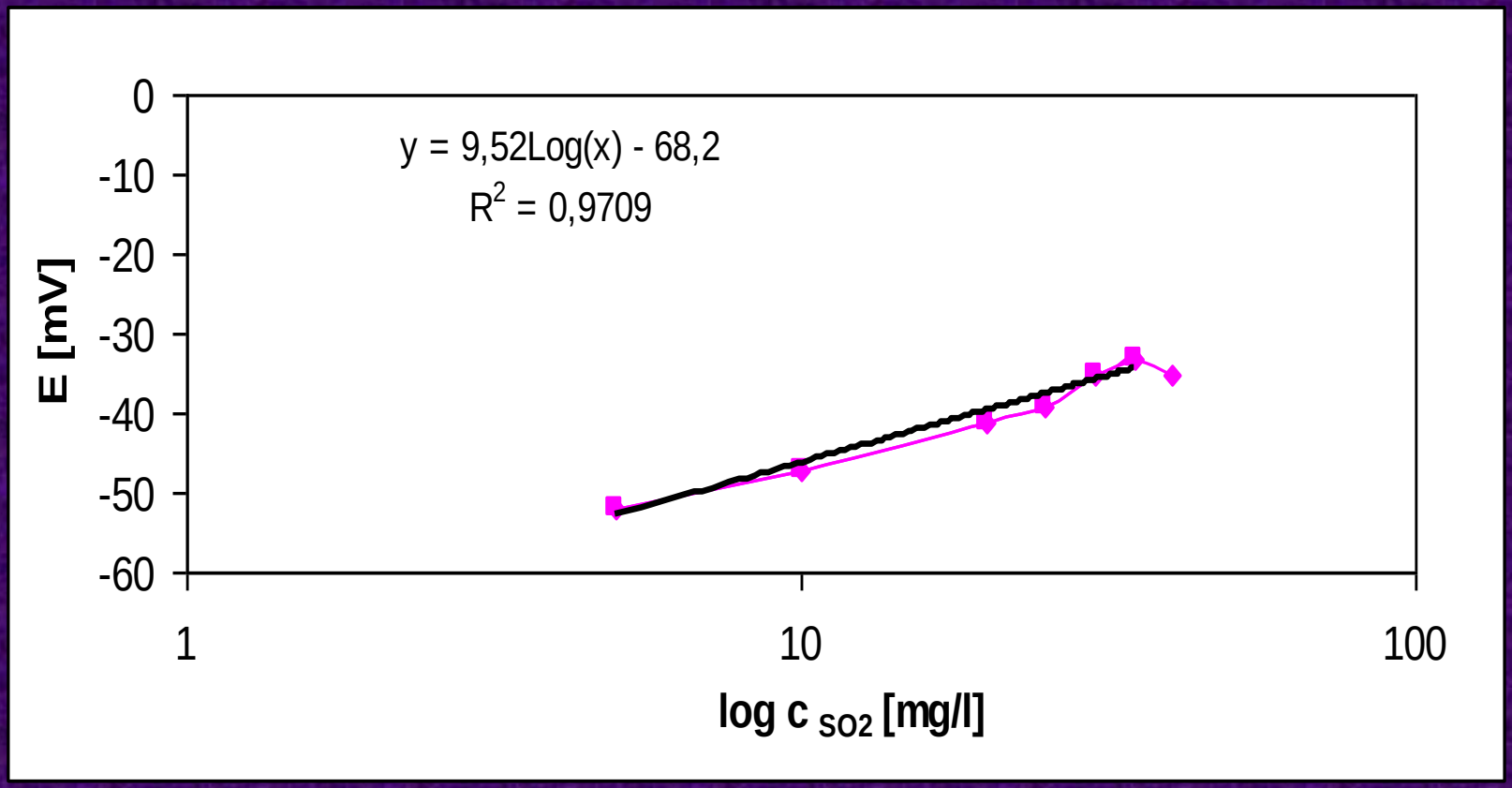


Potentiometric CO_2 electrode

Free SO_2 measurement

→ **Red wines** →**White wines**

5-35 mg SO_2/l 5-25 mg SO_2/l



The Crew

Marin BEROVIČ – FKKT Ljubljana
Aleksandra PIVEC – ZRS Bistra Ptuj
Tatjana KOŠMERL – BF Ljubljana
Mojmir WONDRA – BF Ljubljana
Andrej HOLOBAR – ECHO Sl.Konjice
Keith Racman – Mettler Toledo
Mateja Potočnik – FKKT Ljubljana
Jasna Štrus – BF Ljubljana
Štefan ČELAN – ZRS Bistra Ptuj