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Electromagnetic Fields (0.04 to 0.39) mT effect on cellular growth cycles of *Saccharomyces cerevisiae* wine strains

Efecto de campos electromagnéticos (0.04 a 0.39) mT en los ciclos de crecimiento celular de cepas de vino de *Saccharomyces cerevisiae*

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Abstract.

Low frecuency electromagnetic fields effect (EMF) on growth cycles of yeast *Saccharomyces cerevisiae* wine strains Rv1 and Rhône were studied. A cylindrical coil induced magnetic fields with inductions up to 0,39 mT. Exposure time to EMF varied between (1 – 10) min at 30 °C. The biomass growth were monitored in the reactor culture media (yeast extract + by measurement optical density from (0 to 32) h. The biomass was found by dry weight. After yeast expose to the different EMF, the number of growth cycles decreased from 4 cycles to 2 or 1. However, the biomass production increased almost 50 %. The best biomass production was found at 0.39 mT and 10 min exposure time.

Keywords: Electromagnetic fields, *Saccharomyces cerevisiae*, biomass production, *RV1*.

Resumen.

Se estudiaron los efectos de los campos electromagnéticos (EMF) de baja frecuencia sobre los ciclos de crecimiento de la levadura Saccharomyces cerevisiae cepas de vino Rv1 y Rhône. Una bobina cilíndrica indujo campos magnéticos con inducciones de hasta 0,39 mT. El tiempo de exposición a EMF varió entre (1 - 10) min a 30 ° C. El crecimiento de la biomasa se controló en los medios de cultivo del reactor (extracto de levadura + midiendo la densidad óptica de (0 a 32) h. La biomasa se encontró en peso seco. Después de exponer la levadura a los diferentes campos electromagnéticos, el número de ciclos de crecimiento disminuyó de 4 ciclos a 2 o 1. Sin embargo, la producción de biomasa aumentó casi 50%. La mejor producción de biomasa se encontró a 0,39 mT y 10 minutos de tiempo de exposición.

Palabras clave: campos electromagnéticos, Saccharomyces cerevisiae, producción de biomasa, RV1.

1.-Introduction.

The importance of *Saccharomyces* yeasts to produce alcoholic fermentation and other secondary metabolites of industrial interest have been previously recognized (Sheehan, et al 1999; Clavijo, et al 2010; Morata, et al 2006: Amado-González, et al 2005). The possibility of improving the production of metabolites by the application of electromagnetic methods may be interesting in the development of biotechnology projects. However, the use moderate pulsed electric fields on early stages of *Saccharomyces cerevisiae* yeast suspensions was found to produce irreversible flocculation (El-Zakhema, et al 2006). But, it was found that non-homogeneous static magnetic during fermentation can enhance ethanol productivity (Deutmeyer, et al 2006).

Actually, several research to understand the effects of magnetic fields on *Saccharomyces cerevisiae* has done. Experiments on yeasts exposed to magnetic field at 0.35 and 2.45 mT by 24 and 72 h, found no effect on the growth of *Saccharomyces cerevisiae* (Ruiz-Gómez, et al 2004). However, a study using magnetic fields on *Saccharomyces cerevisiae* at 220 mT by 24 h produced a 2.5 fold increase in biomass (g/L) and 3.4 fold increase in ethanol concentration (Da-Motta, et al 2004). On the other hand, J. Novák (2007) reported that magnetic field decreases the number of yeasts, and slowed down their growth. Recently, it was found that pulse of electromagnetic field of 5.0 kV/cm and 20 □s pulse with on *Saccharomyces cerevisiae* improve simultaneous accumulation of magnesium and zinc ions in the biomass (Pankiewicz, et al 2014). The controversial results suggest that experiments were done by different procedures and were non-parameterized. Recently, a systematic review of published scientific studies on potential effects of EMF shows an urgent need for repetitions of experiments under standard conditions to confirm the presence/absence of effects (Cucurachi, et al 2013).

The aim of the present work is the result of a systematic study (Álvarez-Ovallos et al 2014) the EMF on the growth cycles and biomass production of the yeast *S.cerevisae* RV1 (PTA08) that was the first microorganism from the Spanish type collection obtained by the University of Roviri i Virgili and the *S.cerevisae* Rhône 2056, Lallemand used for the production of wines.

2. Materials and Methods.

A cylindrical coil generated the magnetic fields. The EMF is generated by a Helmholtz cell (10 cm diameter) with 2130 threads of copper wire (2 mm), mounted on a wooden base powered by a transformer (Fig.1). A maximal effective current was 1.9 A at Hz. The samples were exposed and incubated at 30 °C. An incubation time of 72 h at 100 rpm was used. After incubation time, the batch reactor was introduced into the EFM cell. The amplitude of magnetic field induction (B_m) varied between 0.04, 0.21 and 0.39 mT. Yeasts culture was exposed to EMF 1, 5 and 10 minutes.

Commercial yeast *S. cerevisiae* Rhône 2056, Lallemand and *S.cerevisae* RV1 (PTA08) were used. The activation was done by inoculating the lyophilized of *S. cerevisiae* strains 1% (w/v) in a liquid culture medium (10% glucose, 0.2% KH_2PO_4, $(NH_4)_2SO_4$, 0.3%, $MgSO_4.7H_2O$ 0.1%, 0.4% and yeast extract 0.36% peptone) The strain was incubated at 28 °C by 48 hours. The liquid medium was previously sterilized at 121 °C, and the pH was adjusted with sterile 0.1N HCl. The growth conditions of the variety RV1 were 30 °C at pH 5.0.

Biomass was obtained from each flask by centrifuged and washed with sterile distilled water abundant finally taken to an oven dried at 105 °C until constant weight.

The determination of yeast growth curves was done by a Spectrophotometer Hach DR 2010 to measure the optical density (OD) at wavelength of 600 nm. The calibration curve at OD. 600 and the counting of the number of CFUs were done. Every hour optical density (OD) was measured and the number of cells was calculated from the calibration curve. The absorbance values were correlated to dry biomass (g/L), calculated from a linear regression equation (y= 0.6974x + 3.766), by the relation (1):

$$\text{biomass}\left(\frac{\text{g}}{\text{L}}\right) = \frac{3.766-\text{absorbance}}{1.638} \qquad (1)$$

Samples of 1.5 mL were collected by triplicate at an interval of 1 h for determination of biomass up to 36 h.

3. Results and Conclusions

The ODs measured during the exposure of the yeast culture were compared with the control ones. The yeast cultures Rv1 and Rhône were exposed to the EMF (f=50 Hz, B_m= 0.04, 0.21 and 0.39 mT and t = 1, 5 and 10 min) by using a design 3x3. For Rv1, at 0.29 mT the OD for all exposed samples was found higher than control for all exposed time. But at 0.39 mT the OD was lower than the control for all exposed samples as shown in Fig 2 (A). However, OD for all exposed samples of Rhône compared with control one showed at 0.39 mT were higher at all exposed times as shown in Fig.2 (B).

Novák et al (2007) found inhibiting effects on the growth of the yeasts *Saccharomyces cerevisiae* after their exposure to magnetic fields (Bm≤10 mT, t≤60 min, f=50 Hz. An inhibition was observed immediately after putting the yeast culture into the magnetic fields. Also Ruiz-Goméz (2004) reported not alterations in the growth of *S. cerevisiae* at B_m (0.35 and 2.45 mT) by exposure of 24 h and 72 h. However, Deutmeyer et al(2011) found an 8% increase in peak ethanol concentration by non-homogeneous static magnetic field. Even Gos et al (2000) found no evidence on the mutation and the stress levels of yeast (Saccharomyces cerevisiae) in exposure at 900 MHz. The experiments of Crouzier et al (2009) on membrane fluidity diminutions after exposure in all the conditions suggested an increase of the free radical production in the intra cellular compartment but no effect on the yeast vitality was found. Recently, Shu-Wei et al (2014) reported effects on Yeast ZSM-001 growth and cell membrane permeability by microwave exposure dose and time. At the optimal dose of 1.6 W/g for 40~120 s, yeast growth rate increased up to the maximum at 1.6 W/g for 120 s.

The comparative relation values (r.v.) of exp./control for all experiments with the standard deviation are shown in Fig. 3 for Rv1 and Rhône (Table 3). The results of Rv1 show at B_m = 0.04 mT r.v.<1 and increase between 15 to 30 h. For Rhône at B_m = 0.04 mt, we found that r.v. > 1 until 30 h. The differences of r.v. due to the exposure time are not relevant. At B_m = 0.21 mT, the strains Rv1 and Rhone show r.v.> 1 for exposed time of 1 and 5 min. But to 10 min exposed time r.v. <1 for both strains. This results shows that the EMF is not constant. And it may be the reason of the controversy about the results.

On Fig. 3 at B_m = 0.39 mT is found that values of r.v. <0.18 for Rv1, this would imply that the inhibition effect. However, exposure times Rhone 5 and 10 min r.v. > 1 would imply a stimulatory effect on growth until 35 h, but at 1 min r.v. is approximately 1 and decreases above 35 h.

Stimulation or inhibition of cellular processes by EMF are considered of interest in cell biology and biotechnology, not only the establishment of the basic mechanisms of this interaction, but also their practical applications. Depending on the form and the values of x, are different effects. Inhibition or stimulation of vital activity of microorganisms can be achieved by selecting the appropriate parameters of intensity, frequency, length, or exposed time to the EMF (Kovacs, et al 1997).

The studies of early stages of *Saccharomyces cerevisiae* yeast suspensions by Zakhema et.al. [18] showed damage in moderate pulsed electric fields using a 7.5 kV/cm electric field strength. And Loghavi et al (2008) found that pure sinusoidal waves at 45 and 60 Hz reduced the lag phase most significantly of *Lactobacillus acidophilus*.

On Fig. 4 the values of (□Growth/□T) against time are found for both strains Rv1 (A) and Rhône (B). Control culture for Rv1 shows two maximums at 11 and 28 h. At B_m =0.04 mT and exposed time of 1 min, two maximums growing cycles at 5 and 14 h are found. But at 5 and 10 min just one maximum is observed. Besides yeast cultures stop growing around 22 h. For Rhône strain, control culture shows two maximums around 5 and 12 h. And at 1 min exposed time, It is found one maximum. Two maximums can be observed at 5 min exposed time, but it is not a clear maximum at 10 min exposed time. On Fig. 4 at B_m= 0.21 mT and 1 min exposed time, Rv1 yeast culture show two maximums growing cycles at 8 and 15 h but stop growing around 24 h. For Rhône yeast culture at 10 min and around 9 h is found an important growing cycle. With the exception at 5 min exposed time and around 21 h, for the other experiments it is not observed a clear maximum. On Fig. 4 at Bm = 0.39 mT for Rv1 yeast cultures not growing cycles are found and cell death is found around 18 h. However, for Rhone yeast cultures for 5 and 10 exposed time, two growing cycles are observed. Death cell is around 36 h.

The results suggest that it is not necessary to use stronger fields, to produce changes in the mechanisms related to growth cycles of the external fields on organisms. The use of longer exposure time not necessary produces an increase of growth cycles. Zhang et al (2002) found that extremely low frequency pulsed-gradient magnetic field may be able to inhibit the growth and division of malignant cell. How low EMF affects growth cycles is still a matter of discussion and controversy. But the results suggest the number of growing cycles decrease after EMF yeast culture exposition and cell death is found before than control culture. Even it is not possible to find a standard answer. Fojt et al (2004) showed that viability decreases with longer exposure time and/or higher induction B_m for all strains, but the quantity of the effect is strain-dependent. Our results

On the other hand it is important to test the production of wine and its quality with yeast culture after EMF exposure.

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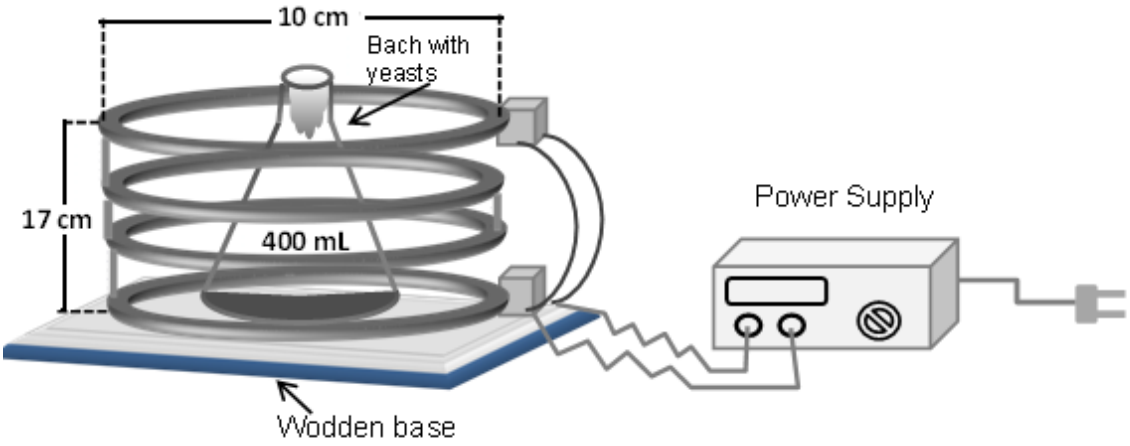
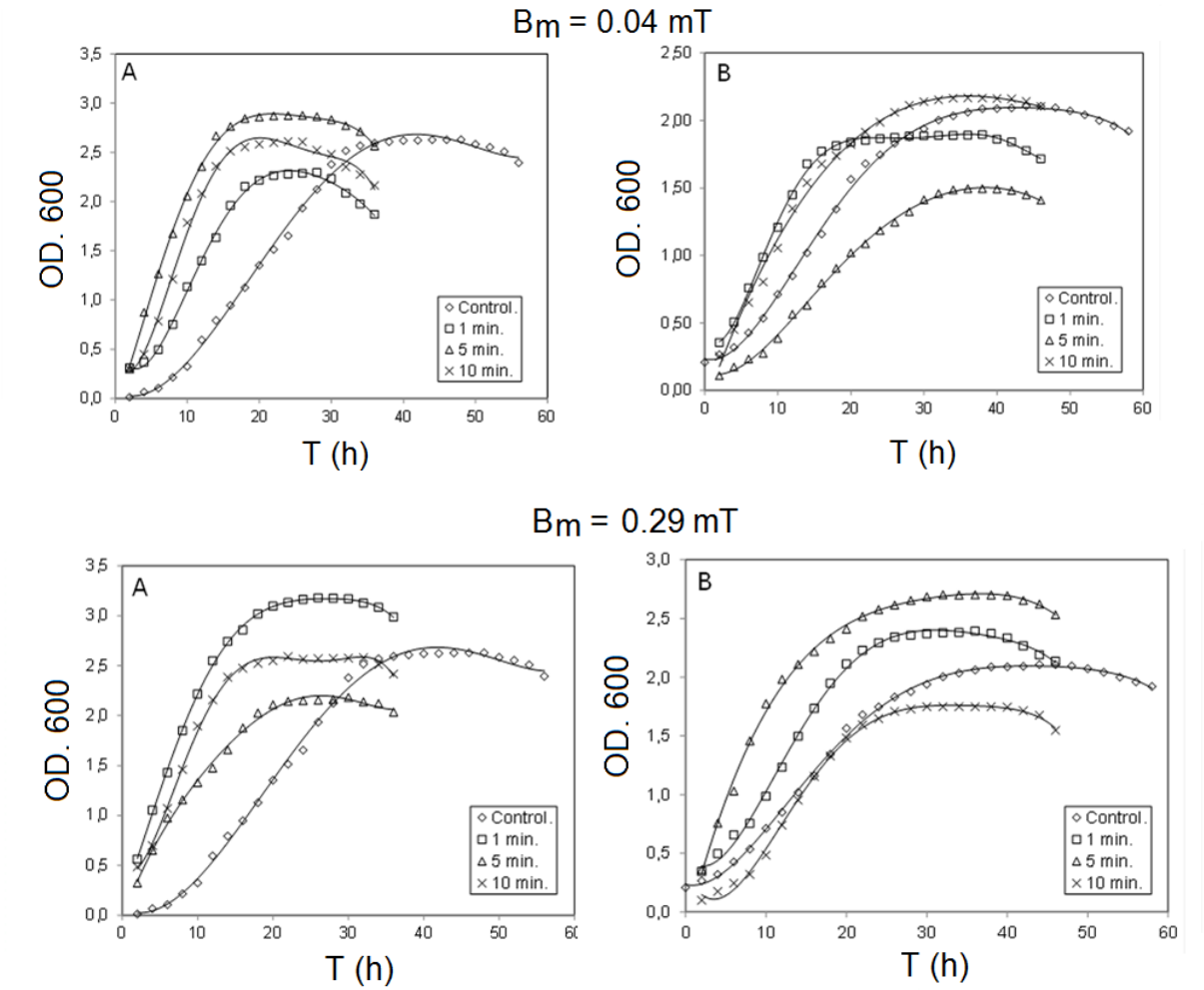


Fig. 1. Cylindrical coil used for the generation of electromagnetic fields.



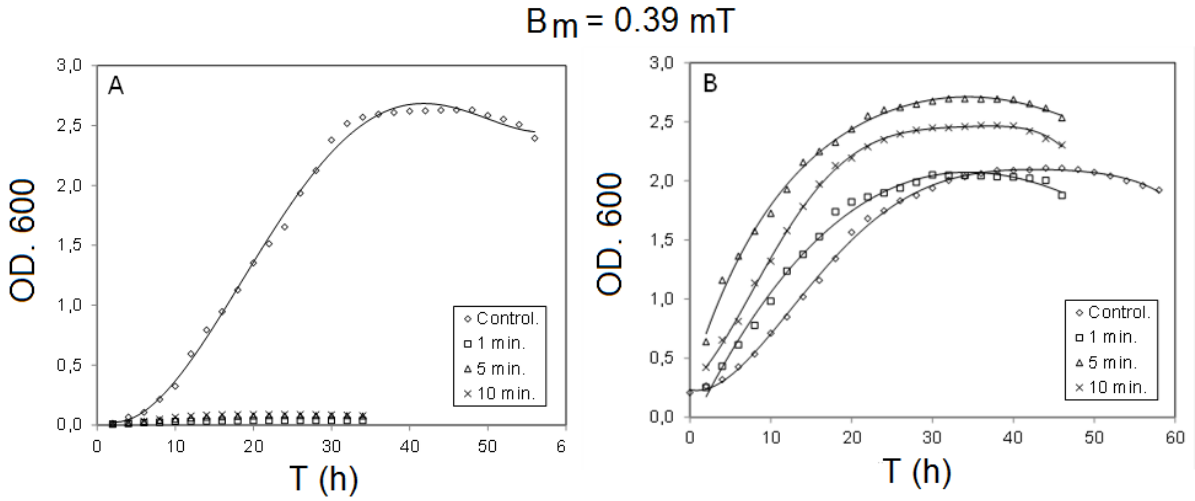
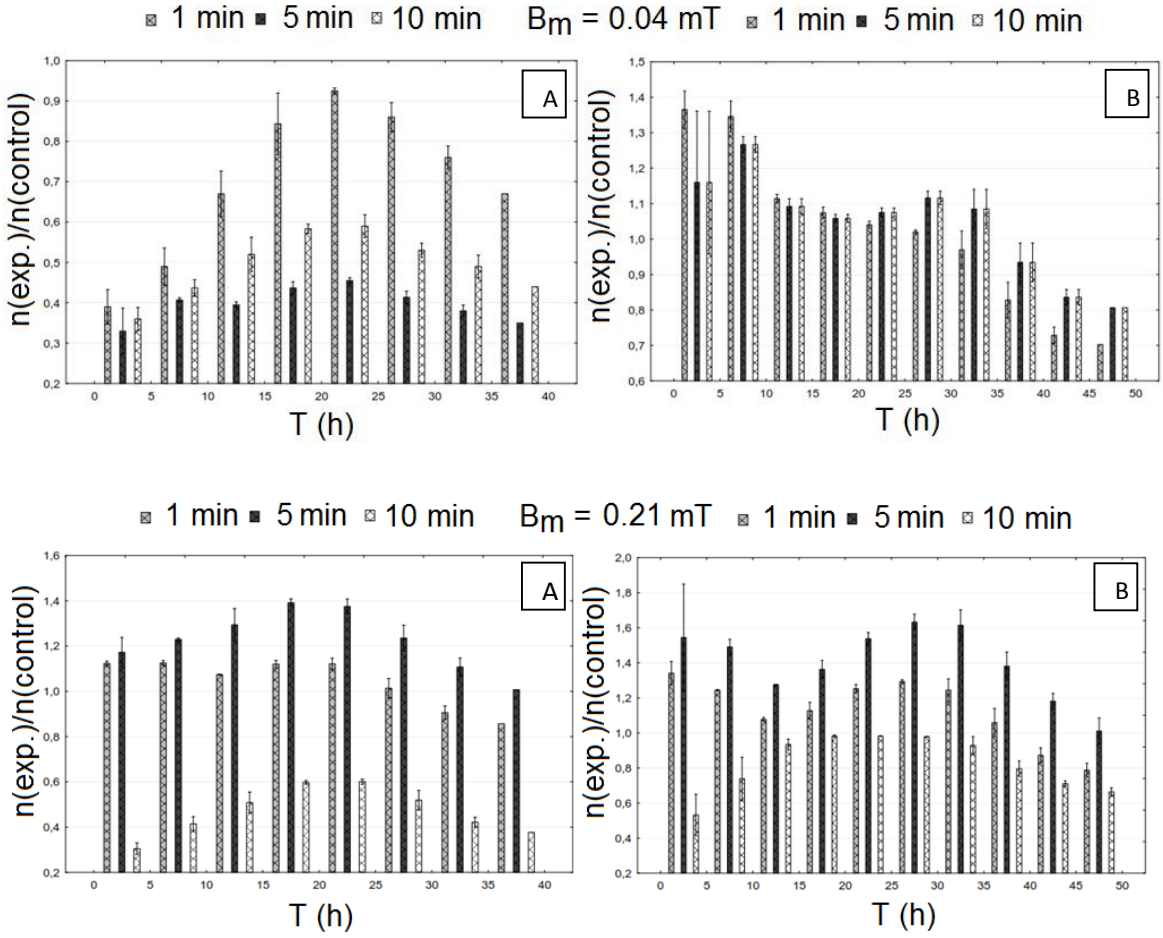


Fig. 2. Values of OD620 for the yeast culture of A: Rv1 and B: Rhône. Magnetic field induction varied from 0.09 to 0.39 mT at (1, 5 and 10 min) exposed time.



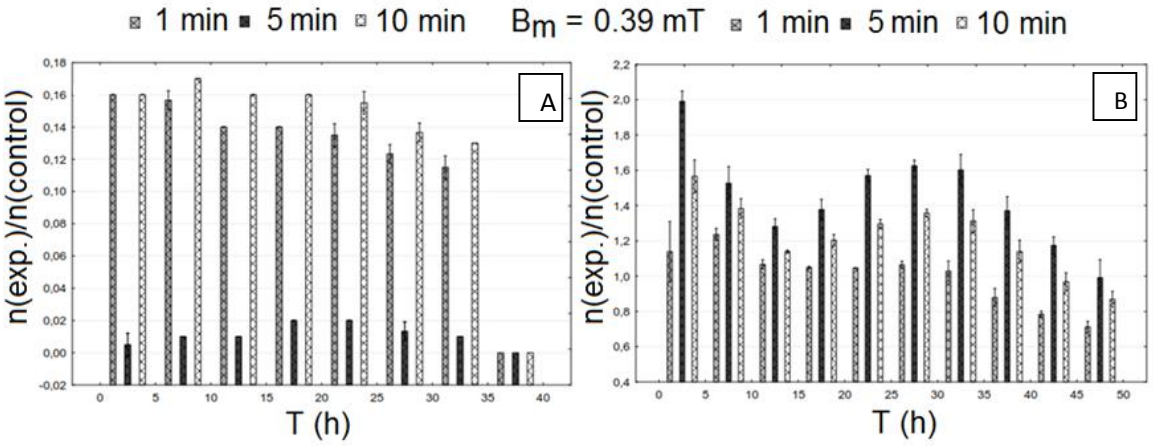
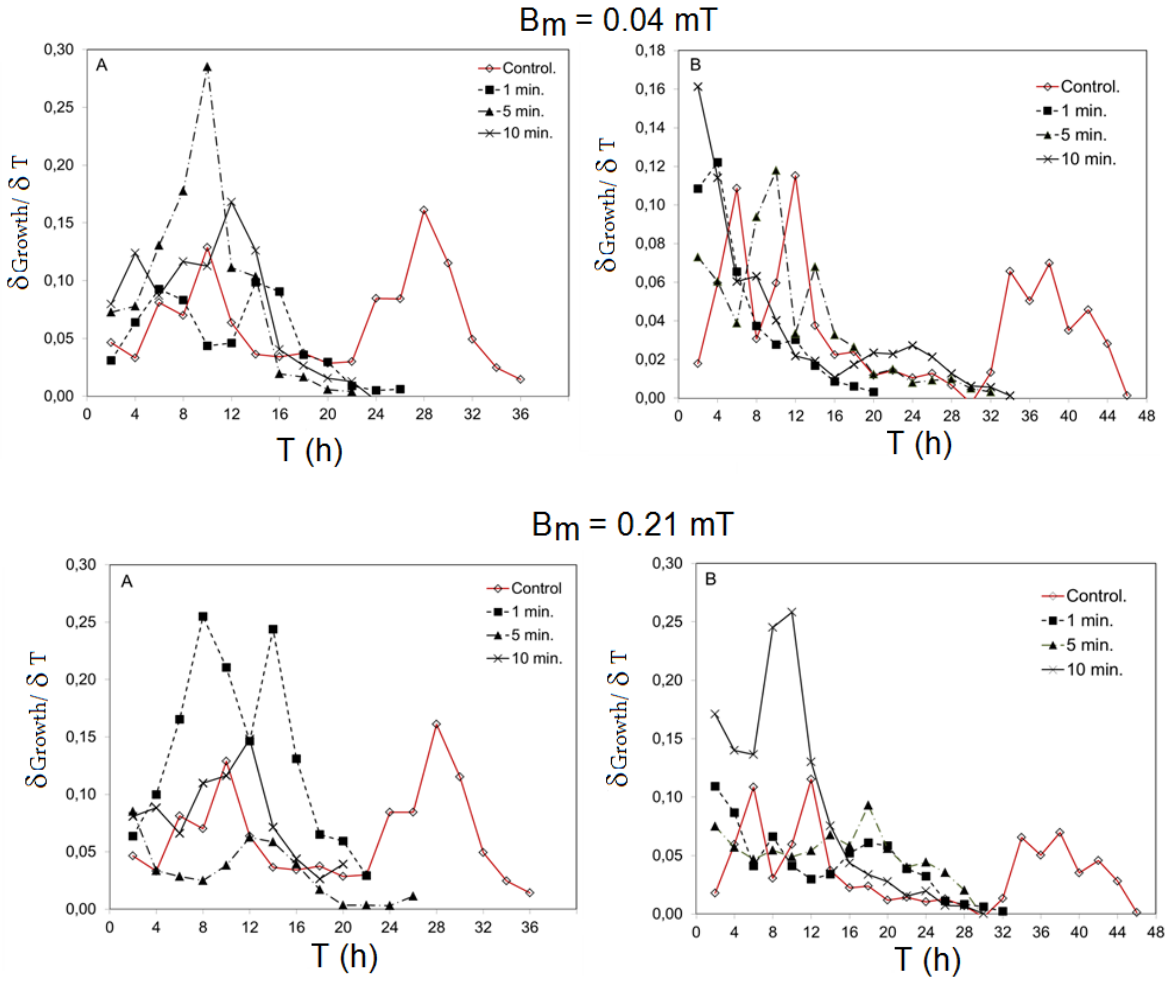


Fig. 3. Relative values of the number of yeasts: A. Rv1 (exp. /control) and B. Rhône (exp. /control). Magnetic field induction varied from 0.09 to 0.39 mT at (1, 5 and 10 min) exposed time.



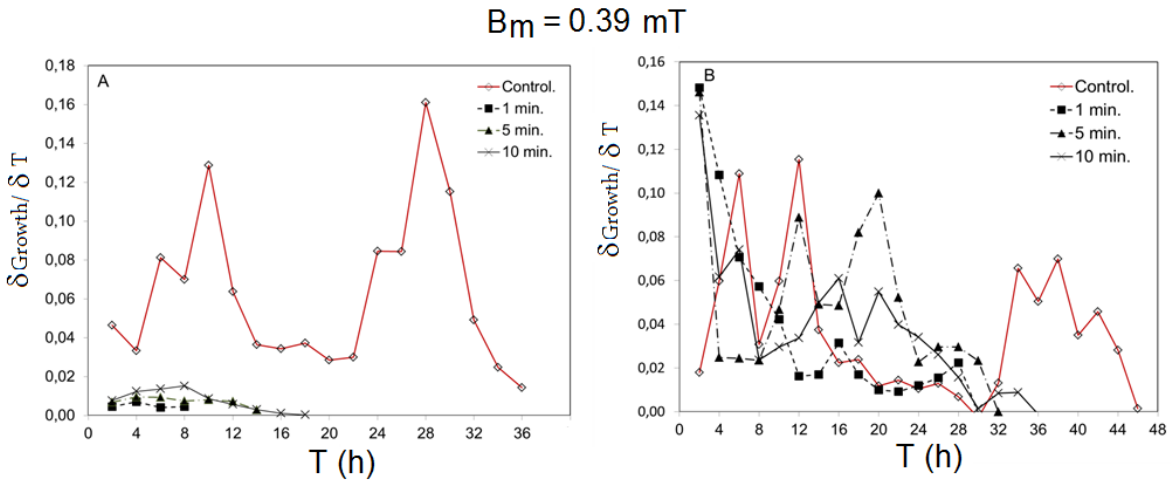


Fig. 4. First derivate of log (growth) with time: A. Rv1 and B. Rhone. Magnetic field induction varied from 0.09 to 0.39 mT at (1, 5 and 10 min) exposed time.

Table 1. The ratio n(exp.)/n(control) of Rv1 in yeast cultures after magnetic exposure B_m (0.04, 0,21 and 0.39mT) at (1, 5 and 10 min), 18 experiments

0.04 mT			0.21mT			0.39 mT		
1min	5min	10min	1min	5min	10min	1min	5min	10min
1.13±0.03	1.13±0.02	0.29±0.08	0.36±0.04	0.29±0.03	0.34±0.03	0.16±0.07	0.00±0.01	0.16±0.01
1.12±0.06	1.22±0.03	0.32±0.02	0.42±0.03	0.37±0.02	0.38±0.01	0.16±0.03	0.01±0.02	0.16±0.02
1.13±0.04	1.24±0.02	0.38±0.03	0.45±0.02	0.41±0.02	0.42±0.01	0.16±0.03	0.01±0.03	0.17±0.02
1.13±0.03	1.23±0.01	0.41±0.12	0.48±0.02	0.41±0.02	0.43±0.03	0.16±0.02	0.01±0.01	0.17±0.11
1.11±0.06	1.23±0.04	0.45±0.04	0.54±0.05	0.40±0.03	0.46±0.03	0.15±0.05	0.01±0.03	0.17±0.03
1.08±0.04	1.24±0.03	0.48±0.03	0.63±0.02	0.39±0.02	0.49±0.04	0.14±0.03	0.01±0.02	0.16±0.02
1.07±0.05	1.34±0.04	0.54±0.04	0.71±0.04	0.40±0.03	0.55±0.03	0.14±0.04	0.01±0.02	0.16±0.02
1.10±0.03	1.37±0.01	0.59±0.02	0.76±0.02	0.42±0.02	0.57±0.03	0.14±0.04	0.02±0.02	0.16±0.06
1.13±0.02	1.41±0.02	0.60±0.01	0.86±0.02	0.44±0.01	0.59±0.01	0.14±0.03	0.02±0.02	0.16±0.02
1.13±0.03	1.39±0.04	0.60±0.04	0.91±0.04	0.45±0.03	0.59±0.05	0.14±0.04	0.02±0.02	0.16±0.02
1.14±0.05	1.40±0.07	0.61±0.06	0.93±0.06	0.46±0.04	0.61±0.04	0.14±0.08	0.02±0.01	0.16±0.01
1.10±0.13	1.35±0.02	0.59±0.02	0.92±0.03	0.45±0.02	0.57±0.04	0.13±0.05	0.02±0.03	0.15±0.09
1.06±0.01	1.30±0.02	0.57±0.03	0.90±0.07	0.43±0.01	0.55±0.03	0.13±0.03	0.02±0.02	0.14±0.02
1.01±0.03	1.22±0.02	0.51±0.02	0.85±0.03	0.41±0.02	0.52±0.04	0.12±0.04	0.01±0.02	0.14±0.02
0.98±0.04	1.19±0.03	0.48±0.03	0.83±0.05	0.40±0.03	0.52±0.03	0.12±0.05	0.01±0.04	0.13±0.04
0.93±0.03	1.14±0.03	0.44±0.05	0.78±0.11	0.39±0.04	0.51±0.04	0.12±0.03	0.01±0.03	0.13±0.02
0.89±0.01	1.08±0.02	0.41±0.02	0.74±0.03	0.37±0.01	0.47±0.11	0.11±0.02	0.01±0.02	0.13±0.01
0.86±0.08	1.01±0.10	0.38±0.12	0.67±0.07	0.35±0.09	0.44±0.12			

Table 2. The ratio n(exp.)/n(control) of Rhône in yeast cultures after magnetic exposure B_m (0.04, 0,21 and 0.39mT) at (1, 5 and 10 min), 18 experiments.

0.04 mT			0,21 mT			0.39 mT		
1min	5min	10min	1min	5min	10min	1min	5min	10min
1.33±0.01	1.02±0.12	1.02±.011	1.29±0.03	1.33±0.07	0.45±0.02	1.02±0.03	1.95±0.09	1.50±0.08
1.40±0.07	1.30±0.06	1.30±0.08	1.39±0.04	1.76±0.05	0.62±0.04	1.26±0.06	2.03±0.06	1.63±0.05
1.34±0.03	1.24±0.01	1.24±0.02	1.24±0.05	1.51±0.04	0.62±0.05	1.20±0.04	1.60±0.03	1.37±0.03
1.39±0.02	1.29±0.02	1.29±0..02	1.25±0.04	1.52±0.02	0.73±0.03	1.27±0.02	1.55±0.02	1.44±0.03
1.30±0.04	1.27±0.05	1.27±0.03	1.24±0.05	1.44±0.04	0.87±0.04	1.24±0.05	1.42±0.06	1.33±0.04
1.12±0.05	1.11±0.06	1.11±0.04	1.08±0.04	1.27±0.05	0.91±0.06	1.08±0.05	1.25±0.05	1.15±0.06
1.11±0.02	1.08±0.01	1.08±0.02	1.07±0.03	1.28±0.04	0.96±0.03	1.05±0.03	1.31±0.02	1.13±0.02
1.09±0.02	1.07±0.02	1.07±0.01	1.08±0.02	1.31±0.02	0.98±0.04	1.04±0.02	1.33±0.01	1.17±0.03
1.07±0.04	1.05±0.03	1.05±0.04	1.12±0.05	1.36±0.04	0.98±0.04	1.05±0.04	1.36±0.02	1.21±0.03
1.06±0.11	1.05±0.02	1.05±0.01	1.18±0.03	1.42±0.02	0.99±0.02	1.05±0.02	1.44±0.02	1.23±0.04
1.05±0.09	1.07±0.07	1.07±0.08	1.23±0.06	1.51±0.05	0.98±0.07	1.05±0.06	1.54±0.07	1.28±0.05
1.03±0.02	1.08±0.09	1.08±0.02	1.27±0.03	1.56±0.02	0.98±0.02	1.05±0.02	1.59±0.02	1.31±0.03
1.02±0.04	1.10±0.05	1.10±0.03	1.29±0.05	1.59±0.04	0.98±0.06	1.05±0.06	1.60±0.04	1.34±0.04
1.01±0.03	1.11±0.01	1.11±0.04	1.29±0.03	1.63±0.02	0.98±0.02	1.06±0.02	1.62±0.02	1.36±0.03
1.02±0.08	1.14±0.03	1.14±0.01	1.30±0.03	1.68±0.03	0.98±0.04	1.09±0.01	1.66±0.02	1.38±0.12
1.01±0.05	1.12±0.06	1.12±0.04	1.29±0.03	1.68±0.02	0.96±0.02	1.07±0.01	1.66±0.01	1.36±0.02
0.93±0.06	1.05±0.05	1.05±0.05	1.20±0.07	1.55±0.05	0.89±0.06	0.99±0.05	1.54±0.03	1.27±0.04
0.88±0.03	0.99±0.04	0.99±0.02	1.14±0.03	1.47±0.03	0.84±0.05	0.94±0.04	1.46±0.03	1.21±0.06
0.82±0.09	0.92±0.05	0.92±0.03	1.05±0.05	1.37±0.04	0.79±0.04	0.87±0.03	1.36±0.02	1.13±0.02
0.78±0.02	0.89±0.01	0.89±0.02	0.99±0.02	1.31±0.02	0.76±0.03	0.84±0.01	1.30±0.01	1.08±0.02
0.74±0.07	0.85±0.05	0.85±0.08	0.90±0.08	1.21±0.06	0.72±0.07	0.80±0.05	1.21±0.03	1.00±0.03
0.71±0.06	0.82±0.14	0.82±0.06	0.84±0.03	1.15±0.03	0.70±0.04	0.77±0.03	1.14±0.03	0.93±0.05
0.70±0.06	0.81±0.06	0.81±0.04	0.82±0.05	1.06±0.04	0.68±0.04	0.73±0.04	1.06±0.02	0.90±0.02
			0.76±0.09	0.96±0.06	0.65±0.11	0.69±0.13	0.92±0.09	0.84±0.05

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