

Influence of Static Magnetic Fields on *S. cerevisiae* Biomass Growth

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ABSTRACT

Biomass growth of *Saccharomyces cerevisiae* DAUFPE-1012 was studied in eight batch fermentations exposed to steady magnetic fields (SMF) running at 23°C ($\pm 1^\circ\text{C}$), for 24 h in a double cylindrical tube reactor with synchronic agitation. For every batch, one tube was exposed to 220mT flow intensity SMF, produced by NdFeB rod magnets attached diametrically opposed (N to S) magnets on one tube. In the other tube, without magnets, the fermentation occurred in the same conditions. The biomass growth in culture (yeast extract + glucose 2%) was monitored by spectrometry to obtain the absorbance and later, the corresponding cell dry weight. The culture glucose concentration was monitored every two hours so as the pH, which was maintained between 4 and 5. As a result, the biomass (g/L) increment was 2.5 times greater in magnetized cultures (n=8) as compared with SMF non-exposed cultures (n=8). The differential (SMF-control) biomass growth rate (135%) was slightly higher than the glucose consumption rate (130 %) leading to increased biomass production of the magnetized cells

Key words: *S. cerevisiae*, biomass, magnetic fields, fermentation

INTRODUCTION

The steady magnetic field stimulation of *Saccharomyces cerevisiae* has been studied looking for to understand induced effects on cellular metabolism. Montenegro (1999) found that cultures exposed to 220mT SMF produced 72% more CO₂ than the unexposed cultures, without a corresponding increase in the glucose consumption. Low intensity (1mT) alternated magnetic fields (AMF) with 60Hz frequency did not induce *S. cerevisiae* mutation, gene conversion or crossing-over (Ager and Radul, 1992), however the levels of the stationary states of specific mRNAs of the yeast cells was altered after AMF of 60Hz and 20μT

intensity exposure during 15 cellular generations (Binniger and Ungvichian, 1996).

The industrial processes of alcoholic fermentation accept 150g/l as the initial mean level value (Miller, 1959) for total reducing sugars (TRS). However, in the *S. cerevisiae* alcoholic fermentation, hexose catabolism follows the anaerobic way until TRS becomes close to 0.1%; at this level the alcohol production is maximal and the fermentative process stops. At a 0.09 to 0.9% glucose concentration in culture medium, *S. cerevisiae* glucose catabolism chooses preferably the anaerobic biochemical way, even in the presence of O₂, leading to glucose repression, due to gene repression which codifies the Krebs cycle

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of respiratory chain (Rettori and Volpe, 2000). A previous work (Montenegro 1999) found in *S. cerevisiae* cultures exposed to 220mT SMF an increased (72%) production of carbonic gas in relation to the control groups. It was later confirmed that at this magnetic flux intensity, the yeast metabolic rate increased (Motta et al, 2001).

Continuous fermentations using 80 to 260 mT SMF intensity flow transversal to the drainage flux resulted gain of 75% in the alcohol production with a 27% glucose consumption growth, compared to the conventional bed percentages (Ivanova et al, 1995). The yeast ethanolic fermentation is known to be directly related to the carbonic gas production (Tadege, et al 1999) so, higher is CO₂ production, greater is the biomass.

In order to observe the influence of magnetic fields on *S. cerevisiae* biomass growth and glucose consumption during a 24 hours. In order to observe the influence of magnetic fields on the *S. cerevisiae* biomass growth and glucose consumption during a 24 h incubation period, a study was designed to measure the dry weight biomass evolution and the glucose concentration changes on non aerated cultures under mechanical agitation, exposed to non-homogeneous 220 mT SMF.

MATERIALS AND METHODS

A fermentation reactor was assembled using two 200ml glass tubes with 40mm diameter, capped with rubber stoppers having a central hole ($\phi = 0.5\text{mm}$) and a separate vent hole ($\phi = 0.5\text{mm}$). These tubes were attached to an acrylic structure, equipped with double mechanical synchronous turning mixers – two acrylic axles passing by the stoppers central hole – which was connected to the framework by a metallic flange and having a pulley fixed near the upper extremity. The axles, provided with four blades attached in the axles lower ends, were driven by an electric motor spinning at 160 r.p.m. . Three identical pulleys link up the motor and axles, with the same rotation, through transmission belts (Fig. 1), allowing a symmetrical homogenizing action in both culture media. The spinning mixers make the cultured cells to grow in the non-magnetized and in the exposed media continuously during 24 h to the SMF. Samples for chemical analyses were withdrawn through the vent of the stoppers.

SMF was generated by two equal assemblies of five NdFeB magnets (Magnet Sales Inc. FL, USA) with 550 mT intensity flow on surface, diametrically placed in the external tube surface. Thus, the five north poles faced attached to the outer wall, faced the south poles of the other assembly, in the opposite wall as shown in Fig. 1.

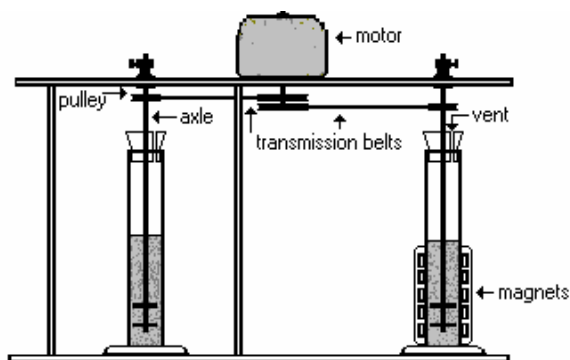


Figure 1 - Schematic assembly of the reactor with 10 magnets attached to the external wall of one tube.

Consequently, every pair of magnets produced a 220 mT flow intensity magnetic field in the center of the tube, between the poles, value obtained by a Teslometer Phywe, model 23.170 (Daedalon Co., USA). The magnetizing glass tube had, thus, five

pairs of magnets assembled in two wooden supports diametrically affixed outside the tubes, separated by a 3.0 cm gap, resulting in a 242mT SMF in the inner wall under each pole. This arrangement generated a non homogeneous 220mT

SMF in the center area of each culture tube with a height of 7 cm with a 22 mT SMF gradient along the tube radius, between every two opposed magnets.

The cultured *S. cerevisiae* yeast strain, DAUFPE-1012, was donated by the Department of Antibiotics of the Federal University of Pernambuco and maintained (mother culture) in an inclined glass bottle at 4°C containing Sabouraud-Agar Dextrose. The pre-inoculation was prepared with cells collected from the mother culture bottle and cultured in two tubes containing each one 10ml of growth medium using yeast extract (5g/L) and glucose (50g/L) (Biobrás Diagnósticos, B. Horizonte, MG-Brazil), during 18 hours at 30°C in a shaker. After the inoculations with nearly 6×10^4 cells in each tube containing 120 ml of growth medium, the fermentations run into the reactor during 24 h at 27°C ($\pm 2^\circ$).

The determination of the cellular growth was done by the culture turbidity, i.e., relating the absorbance value obtained by a LABOMED INC Spectro RS spectrophotometer (S.Paulo, SP, Brazil) at 660nm wavelength, with cells dry weight. The absorbance values were correlated to a cell dry weight (g/L) standard line, obtained by plotting the weight of a known volume of desiccated culture whose cell

number was known against the respective absorbance value, which resulted in the equation 1:

$$y = 1.638x + 0.16921 \quad (1)$$

The equation 1 was calculated from a linear regression of experimental absorbance and biomass averaged values. Extracting the biomass value (x), results the equation 2:

$$\text{Biomass (g/L)} = (0.1692 - \text{absorbance}) / 1.638 \quad (2)$$

The culture acidity was measured by a portable Corning PS-15 pH meter along periods of 2 h in order to maintain it at 4 to 5 to optimize the fermentation process.

The glucose level was evaluated by means of the dinitrosalicylic (DNS) acid method (Miller, 1959). After this measurement, the concentration of glucose was obtained in both cultures by spectrometric comparison with glucose standards. The samples for these analyses were collected every 2 h.

RESULTS

The biomass growth and glucose level values obtained from SMF exposed and unexposed cultures along 24 h are shown in Table 1. The biomass dry weight in both cultures shows roughly the same values during the first 8 h of fermentation.

Table 1 - Averaged biomass (dry-weight) and glucose mean level of non exposed (control) and exposed (SMF) cultures, observed during 24 h (n=8).

Time(h)	Biomass (g/L)		Glucose (g/L)	
	Control	SMF	Control	SMF
0	0.05	0.06	54.02	55.09
2	0.09	0.10	51.53	52.60
4	0.28	0.29	49.04	50.12
6	0.46	0.51	47.37	47.71
8	0.51	0.75	45.70	45.30
10	0.56	1.04	34.00	30.74
12	0.57	1.09	32.91	25.65
14	0.60	1.23	31.82	20.56
16	0.66	1.26	30.76	20.43
18	0.69	1.48	29.69	20.30
20	0.77	1.87	15.95	7.68
22	0.81	2.08	12.23	0.30
24	0.89	2.23	12.34	0.01

The glucose depletion was higher in magnetized cultures. After 8 hours the control cultures consumption was almost stabilized whereas SMF cultures consumed all sugars in medium in 24 h. A statistical analysis by the Student test for one-tailed and non-homocedastic values for the averaged

biomass mean data from both cultures resulted significant at $\alpha = 0.05$ and the correlation between the mean of these data resulted $R = 0.959065$. The analysis of the averaged glucose content data resulted non-significant, using the above described method.

The biomass mean values in Table 1 increased until 4 h of fermentation in both cultures and diverged thereafter. The SMF exposed yeast biomass grown at a higher but the unexposed ones presented a mild growing rate. In fact, the magnetized cultures biomass showed a growing rate 3.7 times superior to that observed in non-magnetized cultures. The biomass value at the fermentation end (24 h) of the SMF exposed cells was 2.5 times greater than the control one. The contrary was observed on the

averaged glucose level data (Table 1), i.e. the sugar depletion in SMF exposed cultures was higher than that in the non-exposed ones, from 8 h to the end of the fermentation. The final mean value of the glucose concentration in magnetized samples was 0.08% of the final non-magnetized cultures residual sugar. The depletion rate the averaged glucose level data in the magnetized cultures was 32.2% greater than the non-exposed ones after a linear regression analysis.

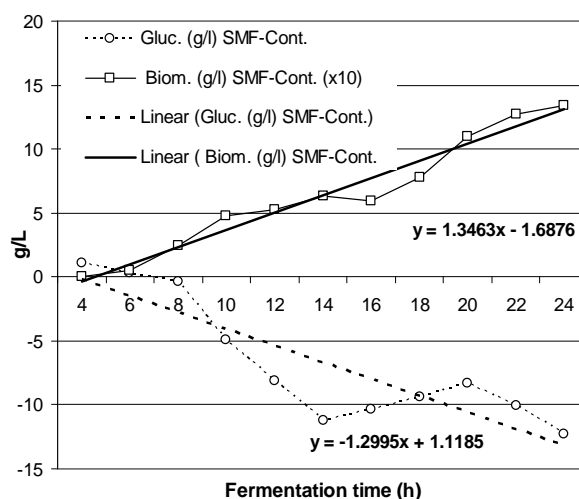


Figure 2 - Biomass (Biom.) and glucose (Gluc.) net values (SMF-Control) regression lines in both the cultures from 4 to 24 h.

Fig. 2 shows the difference between biomass values and glucose content of SMF exposed and control cultures. The regression lines indicated that the net (differential) cell growth and glucose consumption were nearly 1.3 times greater in magnetized cells than in control ones. The pH was corrected three times with alkaline solution in magnetized cultures and one time in control ones.

DISCUSSION

Previous findings (Montenegro, 1999; Motta et al, 2001) showed an increase of the *S. cerevisiae* metabolism after magnetic stimulation. Table 1 showed a greater biomass growth rate of the magnetized cultures starting after 4 h of incubation when the exponential phase of cellular proliferation begun. At the end of fermentation time (24 h), the biomass dry weight in magnetized cultures was consequently higher (2.59 times) than

control ones. Table 1 also showed that glucose concentration values are inversely proportional to the biomass dry weight. Fig. 2 showed that both regression line slopes, from the biomass and glucose data, were synchronic and inverse along all the fermentation cell growth period of time. Moreover, the slopes reveal that SMF exposed cultures biomass production was 1.3 times more accelerated than unexposed cultures, despite the inhibitory effect caused by the presence of ethanol in the growing medium upon *S.cerevisiae* reproduction (Lopes-Dahabada and Sola-Penna, 2001). In addition, the final mass of magnetized cells (Table 1) was more than the double of non-magnetized ones. Thus, to grow faster and more, obviously the SMF exposed cultures consumed proportionally more glucose, finishing the observation time almost with no carbon source in the cultures medium

A statistical non-significance was observed between the glucose averaged data from both cultures and was probably caused by the almost

similar consumption up to 8 h. The SMF exposed cultures glucose presented an overall consumption rate 37% superior to the control cultures. On the other hand, at the end of fermentation the biomass in these cultures was nearly 2.5 times higher, suggesting a more effective glucose-to-biomass conversion.

As reported by Pringle and Hartwell (1998), 220mT SMF accelerates the G1 cyclins-kinase *cdc2* linkage, activating the early passage of the yeast from G1 phase to S phase. This meant that the cellular cycle duration would be smaller than the time necessary for the cellular size duplication, producing smaller cells that would persist until the third generation. This could explain the increment in biomass by the production of a high number of smaller cells in magnetized cultures. Blanchard and Blackman (1994) stated that magnetic fields promote conformation changes in certain proteins, more specifically in enzymes, by means of linked specific ions (magnesium, manganese, calcium and iron) as specific cofactors, beginning the performance of those enzymatic processes, increasing its potentiality. According to Bialek et al (1989), these transitional changes generate electromagnetic fields which suffer influence of SMF, causing a change inside of the enzymatic process, inducing an improvement of enzymatic activity.

Table 1 shows yet an augmentation of biomass and corresponding reduction in glucose. Furthermore, it shows a bifurcation on data progression after 4h and 8h, respectively in both culture media, i.e., near the 2nd and 3rd cell generation. This indicated a modification in the growing profile, signifying a higher glucose uptake, leading to a greater rise in cells mass. In addition, at 18h of fermentation the glucose consumption was much enhanced – so as the biomass synthesis – pointing to the appearance of a late cell lineage, more effective in the glucose-to-biomass conversion. These findings were explained by Blanchard and Blackman (1994) who demonstrated that magnetic fields promote conformation changes in certain proteins, more specifically in enzymes, by means of linked specific ions as the magnesium, manganese, calcium and iron, as specific cofactors, enhancing the performance of those enzymatic processes and increasing its potentiality.

The very low final averaged levels of glucose in SMF exposed cultures (almost zero) confirmed the high metabolic rate previously observed (Motta et

al, 2001) as a manifest magnetic field effect. This was supported by the greater acidity correction interventions in magnetized cultures.

Fig. 2 showed that the regression lines point to a high incremental rate in the SMF exposed cells, regarding the biomass growth and the glucose consumption after 4h of fermentation. Its slopes showed that in SMF exposed cells the net biomass growth rate was nearly 135% faster than those of unexposed cells, whereas the glucose depletion rate was roughly 130% superior do the control cultures rate during the exponential phase of cell growth. PEMF radiation induced a biomass augmentation in magnetized cultures that should continue beyond 24 hours (see regression line in Fig. 2), if was provided a higher glucose content in culture media. As the biomass synthesis rate was superior to the glucose consumption rate in magnetized cultures, mutagenic factors probably increased the cytosolic enzymes activity, enhancing the biomass production gain in these fermentations. This conclusion deserves further studies to explain its mechanisms.

RESUMO

O crescimento da biomassa da *Saccharomyces cerevisiae* DAUFPE-1012 foi estudado em oito bateladas de fermentação, cada uma exposta aos campos magnéticos contínuos (CMC), à 23°C (\pm 1°C), durante um período de 24 horas em um reator duplo com agitação sincrônica. Em cada batelada, um tubo foi exposto ao CMC, com 220mT de intensidade de fluxo, produzidos por ímãs de NdFeB fixados diametralmente opostos (N para S) em um tubo do reator de fermentação. Em outro tubo, sem ímãs, a fermentação ocorreu nas mesmas condições. O crescimento da biomassa nas culturas (extrato de fermento + glicose 2%) foi monitorado através de espectrometria e correlacionado ao peso seco de levedura. A concentração de glicose nas culturas foi monitorada a cada duas horas e o pH foi mantido entre 4 e 5. Como resultado, a biomassa (g/L) aumentou 2,5 vezes nas culturas magnetizadas (n=8) quando comparadas com as culturas não expostas (n=8). A taxa de crescimento diferencial (CMC-controle) da biomassa (135%) foi levemente maior que a taxa de consumo de glicose (130 %) sugerindo um ganho no processo

de produção de biomassa nas células magnetizadas.

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