Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 117, 119—123, 2009

UDC 663.14:661.722:582.282.23
DOI:10.2298/ZMSPN0917119G

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MAGNETICALLY ALTERED ETHANOL FERMENTATION CAPACITY OF *SACCHAROMYCES CEREVISIAE*

ABSTRACT: We studied the effect of static magnetic fields on ethanol production by yeast *Saccharomyces cerevisiae* 424A (LNH-ST) using sugar cane molasses during the fermentation in an enclosed bioreactor. Two static NdFeB magnets were attached to a cylindrical tube reactor with their opposite poles (north to south), creating 150 mT magnetic field inside the reactor. Comparable differences emerged between the results of these two experimental conditions. We found ethanol productivity to be 15% higher in the samples exposed to 150 mT magnetic field.

KEYWORDS: ethanol production, magnetic fields, *Saccharomyces cerevisiae*

INTRODUCTION

Process of fermentation of sugar into ethanol is one of the earliest biological reactions empirically undertaken by man. The significance of this process is even greater today than it has ever been, since humanity is constantly reaching for innovative uses of its major product, not only as the agent responsible for the rising of bread dough, or basis of alcohol industry, but also as sustainable biological fuel (Pimentel and Patzek, 2005). However, yeasts' ability to produce ethanol is regulated and constrained by their inability to survive high concentrations of this alcohol due to its toxic effects (Lau and Dale, 2008). Even the most tolerant yeast strains can not survive ethanol concentrations above 15% (Morais et al., 1996.). Therefore, during the last few decades, the subject of extensive investigations has been creating the yeast strains which are able to survive higher concentrations of ethanol, but which maintain their fermentative abilities. Some of those experiments involved genetic manipulations, even though the use of modified strains was abandoned since scientists had recognized possible collateral damage caused by unexpected effects on yeasts' metabolism, in case of accidental release into the en-

vironment. After failing to solve this problem by means and methods available to molecular biology, some efforts were employed in finding the solution at the level of quantum biology, utilizing electro-magnetic and static magnetic fields (M a n o l i u et al., 2005). Susceptibility of various microorganisms to electric and magnetic fields was thoroughly studied both *in vivo* and *in vitro* conditions (G a l o n j a and C o g h i l l, 1999).

MATERIAL AND METHODS

Saccharomyces cerevisiae culture was purchased from Albright laboratory, Abergavenny, and prepared for the fermentation. Normally, 1% toluene, 4% ethanol and 0.075% triton X-100 are added for the purpose of permeabilization of the cells. To avoid these permeabilizing supplements, we substituted them with 150 mT magnetic field which was capable of removing positively charged calcium ions and loosening membrane architecture. Subsequently, extra calcium enters the cell from the environment and stimulates cell metabolism. ATP, NAD^+, magnesium and inorganic phosphate were added in order to initiate the ethanol fermentation. The initial sugar concentration was 200 g/l. The pH of the culture medium was kept between 5 and 6.

Fig. 1 — BioFlo III fermentor, New Brunswick, with pH and temperature probes

Fermentation system consisted of one fermentation vessel (7.5 litre BioFlo III fermentor, New Brunswick, Figure 1) containing free cell yeast culture and fermentation medium, two permanent magnets attached to the reactor diametrically opposed, temperature probes and pH sensors. We opted for BioFlo III rather than BioFloo 310, because of greater feasibility in attaching the static magnets of our choice.

We monitored biomass growth of yeast culture by means of optical density correlated to dry cell mass, during sixteen hours of exposure in the reactor. Levels of sugar remained, and the concentrations of ethanol produced were measured every four hours.

RESULTS

At the end of a 16 hour experimental period, cell density and ethanol concentration values in magnetically treated samples and samples that were not exposed to 150 mT magnetic field showed significant differences.

Fermentation aided by static magnetic fields resulted in cell density of about 5.5 g/l with maximum ethanol concentration of 44 g/l. Average productivity was 2.75 g/l per hour, with 71.1 % of utilization of sugars. It was

not a linear process, however. Initial state being zero, suggests that ethanol production sped up four hours after the fermentation process started. At that checkpoint time, it was 6 g/l. The measurement taken after eight hours showed massive increase in ethanol production (19 g/l). After twelve hours of fermentation, we measured 39 g/l of ethanol in the fermentation medium (Chart 1).

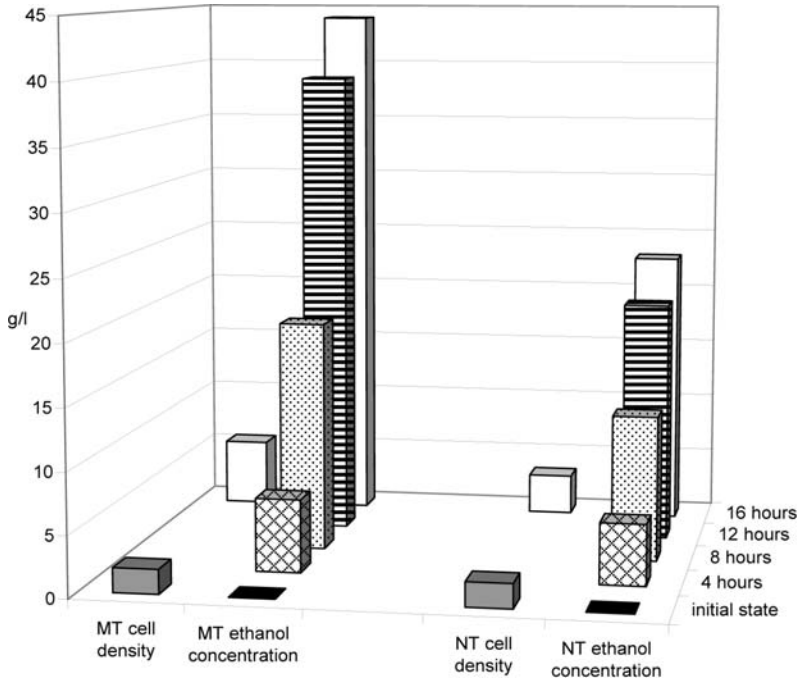

Chart 1 — Changes in cell density and ethanol concentration in magnetically treated samples (MT) and not treated samples (NT), during 16 hours of fermentation

Final cell densities in non-exposed samples were about 3.3 g/l. Ethanol production was not a strictly linear process, measuring 5, 12, 20 and 23 g/l after four, eight, twelve and sixteen hours of fermentation, respectively (Chart 1).

CONCLUSION

Magnetic fields do not only posses the capability of permeabilizing the cells and increasing their metabolic levels, but seem to neutralize bio-feedback mechanism of ethanol saturation, which normally leads to stopping the fermentation process. Some experiments suggest that yeast cells immobilized on Ca-alginate beads retain their ability to produce ethanol during four days (P e r e z et al., 2007). Although these results are encouraging, more investigation needs to be done into the optimum magnetic and/or electro-magnetic fields, comparison between free cell media and immobilized media performances, as well as many other parameters that come into focus as new results emerge.

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МАГНЕТСКИ ИЗМЕЊЕНА ЕТАНОЛ-ФЕРМЕНТАЦИОНА СПОСОБНОСТ *SACCHAROMYCES CEREVISIAE*

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Резиме

Алкохолну ферментацију шећера до етанола човек користи емпиријски од својих најранијих дана. Значај овог процеса данас је још већи, с обзиром на изналажење нових употребних могућности етанола, који у систему базираном на одрживом развоју има велику перспективу као биолошко гориво (Pimentel и Patzek, 2005). Ограничавајући механизам повратне спреге у овом процесу је неспособност квасаца да се одрже у медијуму који садржи високе концентрације етанола. Након покушаја да се методама генетичког инжењерства превазиђе овај проблем, решење би могла понудити квантна биологија, употребом електро-магнетских или статичних магнетских поља (Manoliu et al., 2005, Perez et al., 2007). Ова поља повећавају пермеабилност ћелија квасаца и интензивирају метаболичке процесе, притом повећавајући толерантност квасаца према вишим концентрацијама етанола. Ми смо користили статична магнетска поља 150 mT у

ферментационом систему BioFlo III fermentor, New Brunswick. Добијени резултати указују на значајно повећање ферментативне продуктивности квасаца, као и на повећање њихове толерантности према вишим концентрацијама етанола. Након шеснаесточасовне ферментације, при којој је коришћен слободноћелијски раствор квасца *Saccharomyces cerevisiae*, културе које су биле изложене магнетском пољу 150 mT продуковале су укупно 44 g/l етанола. Продуктивност култура које нису биле експониране овим пољима била је много мања и износила је укупно 23 g/l етанола.