XVII[th] World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

FERMENTERS WITH MAGNETIC FIELDS FOR AGRICULTURE. TECHNOLOGICAL AND PRACTICAL ISSUES

SIANNAH MARIA MAS DIEGO[1], CLARA MARTÍNEZ MANRIQUE[2], FRANCISCO SIMÓN RICARDO[3], AGENOR FURIGO JR[4]

[1] CNEA – AP. 4078 CP. 90400, Santiago de Cuba, Cuba. E-mail: siannah@cnea.uo.edu.cu
[2] CNEA – AP. 4078 CP. 90400, Santiago de Cuba, Cuba.
[3] Universidad de Oriente. Dpt. Agropecuaria. CP. 90400, Santiago de Cuba, Cuba
[4] ENQ/EQA UFSC-Florianopolis – Brazil.

CSBE101205 – Section III: Equipment Engineering for Plant Production

ABSTRACT Magnetic fields have a potential effect on increasing the efficiency of process involving fermentation of microorganisms. Previous experiences with magnetic fields of intensities on the 60 – 100 mT range applied to several fungi employed for agricultural pest control were conducted. Exposure times were between 15 and 30 minutes in all cases. Experimental evidence showed an increment of up to 50 % in the growth of *Trichoderma harzianum*, a broadly employed biocontrol fungi under the influence of magnetic fields. On the growth of *Beauveria bassiana*, coiniadiation was improved up to 20 % after the exposition at magnetic fields lower than 0.1 T for 20 minutes. Results for *Verticillium lecanii* at 80 mT and 30 minutes exposure time showed that fermenting time was decreased to 12 hours. Since magnetic fields of low intensity have no toxicological or mutagenic effect the scale-up of this technology is envisioned. Technical and practical issues are discussed in order to integrate the magnetic devices to the fermenting systems. Main technological considerations were done in order to guarantee a homogenous field distribution on the fermenting equipment. Other aspects that were taken in to account were the portability which can ensure the possibility of using this technique at different production capacities. A technological solution is proposed, considering the diversity of fermenting systems, the required flexibility since biological responses to magnetic stimuli are organisms specific. As a conclusion, magnetic technology applied to fermenters can be a way to improve the final product quality, biomass concentration by diminishing fermenting time and operation costs.

Keywords: fungi, magnetic field, fermentation.

INTRODUCTION Magnetism is a physical phenomenon closely related with electricity. Biomagnetics is a newly developed science aimed to the study of the biological effects of magnetic fields and the magnetic fields around living organisms, as well. (Polk and Postow, 1996; Barnes and Greenebaum, 2006; Binhi, 2001).

Although the interaction of electromagnetic fields with biological systems has been investigated, there are no biophysical mechanisms that can explain many of the observed biological effects of low-level of magnetic fields. Magnetic fields action on biological material is exerted at different levels of organization considering the cellular and molecular complexity of the exposed organism. Proposed mechanisms include effects on currents, direct forces on biomagnetic materials, effects on free radicals, ion cyclotron resonance, charge transfer processes, stochastic resonance, etc. (Brocklehurst and McLauchlan, 1996; Dini and Abbro, 2005).

Yield increment on fermentation systems are one of the main aspects on the bioprocess engineering. Design of processes based on living cells or cellular components are common and there is a wide range of microbial products at the chemical, food and pharmaceutical industry that can make use of a technology for a higher efficiency (Borzani *et al*., 2001). Although many reports of positive effects of magnetic on cellular growing of yeast and fungi are published concerning laboratory studies (Azevedo *et al*., 2003; Stavroulakis, 2003), no attempts have been made for a technology that can be used on commercial bioreactors with the same efficiency.

Since cell growth increment represents the possibility of diminishing fermenting times and production costs, the scale-up is therefore the crucial link in transferring a laboratory scale process to commercial production scale.

MAGNETIC FIELDS EFFECTS ON CULTURE MEDIA Water is a constituent of all living things; it is the environment in which body electrolytes and biomolecules reside and interact, a material of well-known dielectric properties. Many of the physical properties peculiar to water are due to its molecular asymmetry, polar nature, and ability to hydrogen bond, which are all interrelated (Colic, 1999).

In biological materials, water is a solvent for salts, protein, nucleic acids, and smaller molecules. Electrolytes in the form of sodium, potassium, calcium, magnesium, chloride, and other ions play an important role in the function of biological systems. Many vital processes depend on a subtle balance being established between the concentration of electrolytes inside and outside the cell.

Cell membrane is, to a great extent, impermeable to the passive exchange of ions but allows directed movement under physiological control. Magnetic fields can also affect the solubility of salts on water enforcing their transport into the cell (Carbonell and Martínez, 1996).

Lekhtlaan-Tynisson *et al*., 2004 reported that magnetic field applied to bacteria cultures induces changes in the physical properties of liquid water in physiological solution and the induced structural and dynamic rearrangements of extracellular water are transferred to bacterial cells and change their functional activity.

MAGNETIC FIELDS EFFECTS ON MICROORGANISMS Experimental evidence showed an increment of up to 50 % in the growth of *Trichoderma harzianum*, in solid susbstrate fermentation, under the influence of a 60 mT magnetic fields for 15 minutes (Mas-Diego *et al*., 2003). On the growth of *Beauveria bassiana*, conidiation was improved up to 20 % after the exposition at magnetic fields lower than 0.1 T for 20 minutes (Mas-Diego *et al*., 2009).

Results for *Verticillium lecanii* at 80 mT and 30 minutes exposure time showed that fermenting time was decreased in 12 hours (González and Serguera, 2002). Nagy, 2005 reported 10-70 % stimulation on the conidia germination of *Curvularia inaequalis* and *Alternaria alternata* at 0.1-3.5 mT static magnetic field. In all cases the field was applied directly to the organism and not to the culture media.

Mutagenicity testing was performed in four strains of *Salmonella typhimurium* and two strains of *Escherichia coli*. No statistically significant effects were observed between exposed and control groups in any of the genotoxicity tests for magnetic fields up to 1.1 mT (Nakasono, *et al*. 2008).

Mas-Diego *et al*., 2009 studied the application of the magnetic field with field strength less than 0.1 T to *Beauveria bassiana* and *Trichoderma harzianum*. The results showed no differences in the stimulating effect of the magnetic field after the third application in both microorganisms and no mutagenic effect as well.

TECHNOLOGICAL CONSIDERATIONS Process validation implies the establishment of documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics (EMEA, 2001).

Since biological responses to magnetic stimuli are organisms specific, the main aspect to consider is that the employed magnetic device must operate at varying magnetic induction for a required time.

Studies of magnetic effects on fermentation systems are mainly divided for liquid and solid fermentation. On liquid fermentation better results were achieved when applied to both the culture media and the microorganism (González-Morlá *et al*. 1999, Lekhtlaan-Tynisson *et al*., 2004). On solid fermentation magnetic field was mainly applied to the slants prior to the inoculums stage (Mas-Diego *et al*., 2003, González and Serguera, 2002, Mas-Diego *et al*., 2009). In all cases, magnetic induction and exposure time were the main variables of the system.

For liquid fermentation systems the recommended arrange would be to by-pass the culture media for the magnetic device. Solid substrate fermentation implies an inhomogeneity of the fermentation media so the suggested mechanisms would be to apply the magnetic device at the inoculation stage as shown at Figure 1.

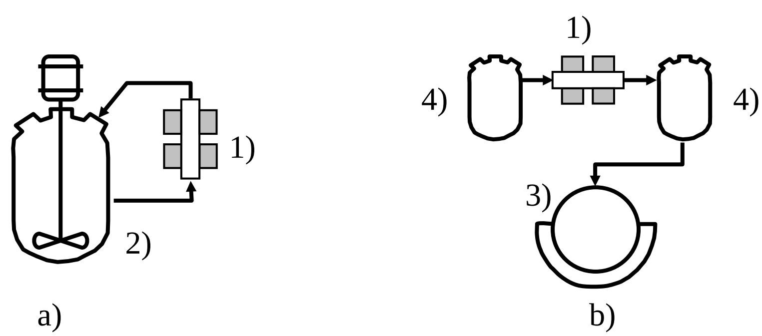

Figure 1. Technological proposal for: a) liquid fermentation b) solid substrate fermentation systems. 1) Magnetic device; 2) Reactor; 3) Solid reactor; 4) Inoculum tanks

The suggested arrangement would be useful for researching purposes, but for production levels, as well.

CONCLUSIONS Magnetic technology applied to fermentation reactors can be a way to improve the final product quality, biomass concentration diminishing fermenting time and operation costs.

REFERENCES

Azevedo, R.B., L.P. Silva, A.P.C. Lemos, S.N. Bao, Z.G..M. Lacava, I. Safarik, M. Safarikova, P.C. Morais. 2003. Morphological study of *Saccharomyces cerevisiae* cells treated with magnetic fluid. IEEE Trans. Magn. 39 (5): 2660-2662.

Barnes, F. S, and B. Greenebaum (eds.). 2006. Handbook of biological effects of electromagnetic fields. Bioengineering and Biophysical Aspects of Electromagnetic Fields. 3rd ed. New York, USA: CRC Press.

Binhi, V.N. 2001. Theoretical concepts in magnetobiology. Electr. Magnetobiol, 20, 43-47.

Borzani, W., W. Schmidell, U. A. Lima, E. Aquarone.2001. Biotecnologia Industrial I. São Paulo, Brazil: Edgard Blücher Ltda.

Brocklehurst, B., and K. A. McLauchlan. 1996. Free radical mechanism for the effects of environmental electromagnetics fields on biological systems. Int. J. Radiat. Biol. 69(1): 3-24.

Carbonell, M.V., E. Martínez. 1996. Modificación de la solubilidad de distintas sales en agua tratada magnéticamente. Química e Industria. 705-707.

Colic, M., and D. Morse. 1999. The elusive mechanism of the magnetic ‘memory’ of water, Colloids and surfaces, A. 154: 167–174.

Dini, L., and L. Abbro. 2005. Bioeffects of moderate-intensity static magnetic fields on cell cultures. Micron. 36(3): 195-217.

EMEA. 2001. Note for guidance on process validation. Available at: www.emea.europa.eu. Accessed 21 January 2001.

González, A, and M. Serguera. 2002. Estudio preliminar sobre la influencia del campo electromagnético en el crecimiento de *Verticillum lecanii* por fermentación en estado sólido. Revista Tecnología Química, 22 (2): 51-61.

González-Morlá, A., S. M. Mas-Diego, M. Campos-Sofía, D. Cabeza-Pullés, Y. Guilarte-Alba, Y. González-Pérez. 1999. Estudio de la cinética de crecimiento en fase sumergida del biocontrolador *Trichoderma viride* bajo la influencia de campos electromagnéticos. Tecn. Qca. 19(1): 67-71.

Lekhtlaan-Tynisson, NP, EB Shaposhnikova, VE Kholmogorov. 2004. The effect of the extremely weak field on the cultures of bacteria *Escherichia coli* and *Staphylococcus aureus*. Biofizika, 49 (3): 519-523.

Mas-Diego, S. 2006. Estimulación del crecimiento de *Trichoderma viride* con empleo de campos magnéticos. MSc. thesis. Fac. Ing. Qca. Universidad de Oriente, Cuba.

Mas-Diego, S. M., C. Martínez-Manrique, F. Simón-Ricardo, A. Furigo Jr. 2009. ‘Memoria magnética’ de sistemas biológicos. Un estudio de casos. III Evento Internacional de Electromagnetismo Aplicado. CNEA. Univ de Ote. Cuba.

Mas-Diego, S., A. Gonzalez-Morla, M. Campos-Sofia, D. Cabeza-Pulles. 2003. Growth of *Trichoderma reesei* on static and dynamic solid substrate fermentation under electromagnetic fields, In: New Horizon on Biotechnology, Roussos, S.; Soccol, C. R; Pandey, A.; Augur, C.; (eds.). Springer, 57-62.

Muniz, JB, M. Marcelino da Motta, A. Schuler, MA da Motta. 2007. Influence of static magnetic fields on *S. cerevisae* biomass growth. Braz. Arch. Biol. Tech. 50(3):515-520.

Nagy, P. 2005. The effect of low inductivity static magnetic field on some plant pathogen fungi. J. Cent. Eur. Agric. 6(2): 167-171.

Nakasono, S., M. Ikehata, M. Dateki, S. Yoshie, T. Shigemitsu, T. Negishi. 2008. Intermediate frequency magnetic fields do not have mutagenic, co-mutagenic or gene conversion potentials in microbial genotoxicity tests. Mutation Research 649: 187–200.

Polk, C., and E. Postow (eds.). 1996. Handbook of biological effects of electromagnetic fields. 2nd ed. New York, USA: CRC Press.

Sahebjamei, H., P. Abdolmaleki, and F. Ghanati. 2007. Effects of Magnetic Field on the Antioxidant Enzyme Activities of Suspension-Cultured Tobacco Cells. Bioelectromagnetics 28:42-47.

Stavroulakis, P. (ed.). 2003. Biological Effects of Electromagnetic Fields. Berlin, Germany: Springer-Verlag.