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Application of Magnetic Immobilized Microorganisms

Ethanol Production by *Saccharomyces cerevisiae*

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Abstract

Magnetic calcium alginate yeast beads, made by incorporation of magnetite or colloidal magnetic liquid, Ferrofluid™, exhibited catalytic behavior similar to that of their nonmagnetic counterparts. The magnetic immobilized preparations' short-term performance, long-term operational stability, and capacity for *in situ* activation were unaffected by the inclusion of magnetic material. The magnetic quality of the alginate beads provides manipulatory advantages.

Index Entries: Magnetic immobilized microorganisms, ethanol production by; immobilized microorganisms, ethanol production by magnetic; *Saccharomyces cerevisiae*, ethanol production by immobilized magnetic; ethanol, production by magnetic immobilized microorganisms; yeast; alginate; Ferrofluid; magnetite.

With the recent rise in gasoline prices, the production of ethanol has attracted considerable attention as a renewable energy source. In the last few years Brazil has begun an intensive ethanol program (*1*). Kierstan and Bucke recently reported the continuous production of ethanol using *Saccharomyces cerevisiae* immobilized in calcium alginate (*2*). The merits for employing immobilized microorganisms for the production of various compounds have been well documented (*3*). However, difficulties arise if suspended matter is present in the substrate medium (e.g., predigested biomass) since separation of the biocatalyst from the fermentation broth in batch processes will be troublesome. Problems will also appear in continuous processes: for example, in packed-bed reactors the suspended matter will plug the reactor. Magnetic immobilized microorganisms would be beneficial in this case be-

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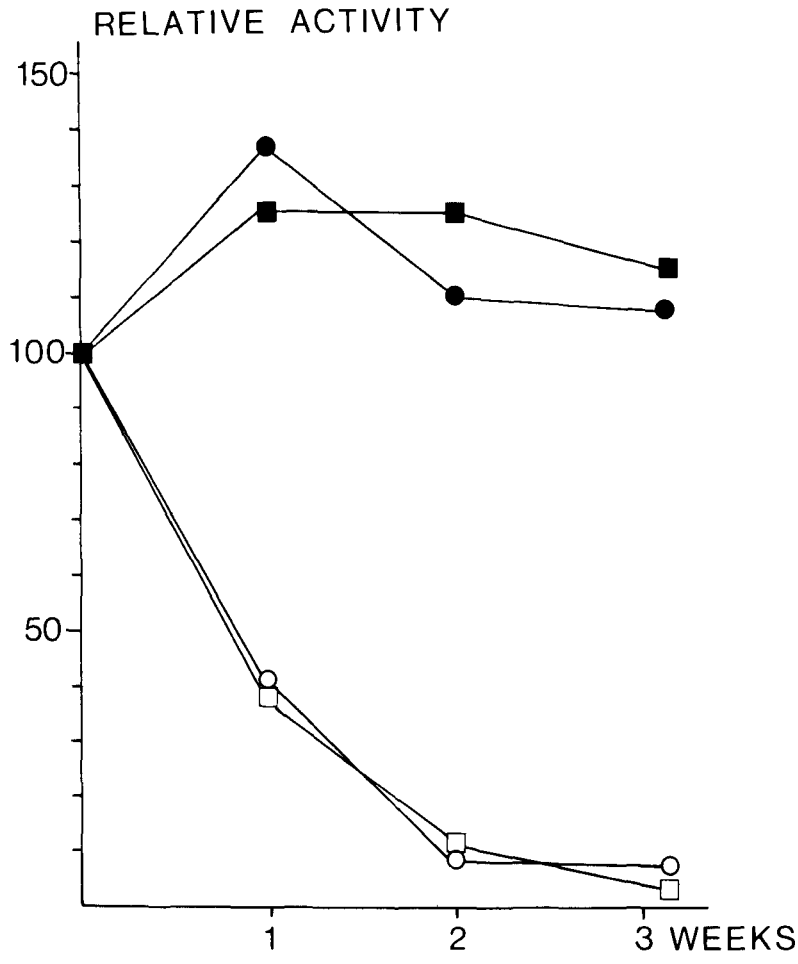

Fig. 1. Long-term operational stability of magnetic and nonmagnetic immobilized yeast beads with and without nutrients: (○) = nonmagnetic, no nutrients; (□) = magnetic, no nutrients; (●) = nonmagnetic, nutrients added; (■) = magnetic, nutrients added.

TABLE I
Activation of Entrapped Yeast

	Nonmagnetic beads		Magnetic beads	
	Cells/g	Activity, m*M* EtOH/min	Cells/g	Activity, m*M* EtOH/min
Prior to activation	4.2×10^7	0.02	4.0×10^7	0.02
After activation	1.8×10^9	4.0	1.7×10^9	3.8

cause they provide a simple and selective means of catalyst retrieval, i.e., by applying a magnetic field to the reactor (*4*). In the current communication we report our comparative studies on magnetic and nonmagnetic yeast preparations.

Various concentrations (0.5–10%) of magnetic material, either magnetite or Ferrofluid™, were included with *Saccharomyces cerevisiae* cells in calcium alginate beads. The experimental details for the preparation and analysis of magnetic and nonmagnetic immobilized yeast have been reported earlier (*5*). In this context mention should be made of recent studies designed to obtain alginate beads stable in phosphate containing media. To this end the beads are treated with high molecular weight polyamines, followed by crosslinking with glutaraldehyde. Alternatively, the polyamines are used in combination with alginate previously modified by carbodiimide or periodate (*6*). All alginate preparations displayed suitable magnetic properties and could be selectively collected by applying a magnetic field. The fermentative ability of all magnetic alginate yeast gels were similar to the nonmagnetic preparations. Only at high Ferrofluid™ concentration (10%) was a reduction in glucose fermenting capacity noticed. A magnetite concentration of 1% was chosen for further study. The long-term operational stability of the magnetic beads was demonstrated in a 3-week fermentation test with and without nutrients and showed analogous behavior to the nonmagnetic preparation, as illustrated in Fig. 1.

In immobilized cell systems, the decrease in activity owing to cell loss and cell death is sometimes the limiting factor of their operational lifetime. It has been shown that immobilized intact living cells are capable of regenerating their activity when nutrients are added (*7*). The ability of magnetic yeast alginate beads to be activated *in situ* was demonstrated in an experiment in which magnetic as well as nonmagnetic alginate beads containing a low concentration of yeast (0.25%) were incubated in nutrient medium (2% glucose, 1% yeast extract, 0.1% $NH_4H_2PO_4$, 0.01% $MgSO_4 \cdot 7H_2O$) under growth conditions for 72 h. The results of such an experiment are shown in Table 1. As can be seen, the increase in activity and cell number was alike for both preparations.

In conclusion, co-immobilization of calcium alginate beads with magnetic material provides means for selective retrieval of the biocatalyst from the reaction medium even if the media contain particulate matter or are highly viscous. The inclusion of magnetic material does not seem to influence the metabolic capacity of the immobilized cells.

References

1. Bazin, M., *Nature* **282,** 550 (1979).
2. Kierstan, M., and Bucke, C., *Biotechnol. Bioeng.* **19,** 387 (1977).
3. Abbot, B. J., in *Advan. Appl. Microbiol.*, D. Perlman, ed., **20,** 203 (1976).
4. Halling, P. J., and Dunnill, P., *Enzyme Microbiol. Technol.* **2,** 2 (1980).
5. Larsson, P. O., and Mosbach, K., *Biotechnol. Lett.* **1,** 501 (1979).
6. Birnbaum, S., Pendleton, R., Larsson, P. O., Mosbach, K., *Biotechnol. Lett.* August, 1981.
7. Larsson, P. O., Ohlson, S., and Mosbach, K., *Nature* **263,** 796 (1976).