

Enzyme Thermistor Control of the Sucrose Concentration at a Fermentation with Immobilized Yeast *

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A broad spectrum of applications shows the advantages and usefulness of using immobilized enzymes in combination with calorimetric techniques for analysis. The enzyme thermistor is based on such a combination and has been designed for routine analysis in clinical chemistry, environmental control and biotechnology.¹

There is a growing need in biotechnology for analysis instruments that can provide reliable,

continuous and specific measurements of metabolites even in crude solutions such as milk, whey and various fermentation broths. In fermentation technology very few measurements can be performed on-line today and the fermentation control is based mainly on registration of variables such as pO_2 , pCO_2 , pH and temperature.

It has recently been shown that the enzyme thermistor can be used for continuous monitoring and control of the conversion of lactose in a bioreactor.² Lactose solutions (*e.g.*, whey) were pumped through a column containing lactase immobilized on Sepharose and the concentration of glucose in the effluent from the reactor was measured with an enzyme thermistor containing co-immobilized glucose oxidase and catalase. The enzyme thermistor signal was used to regulate the flow rate of the pump feeding the lactose solution in such a way that the glucose concentration was kept constant at a preset value even if the lactose concentration or the lactase activity changed.

The present communication describes the use of an enzyme thermistor to control the substrate concentration in a continuously operating model fermenter containing immobilized yeast converting sucrose to ethanol. The enzyme thermistor column

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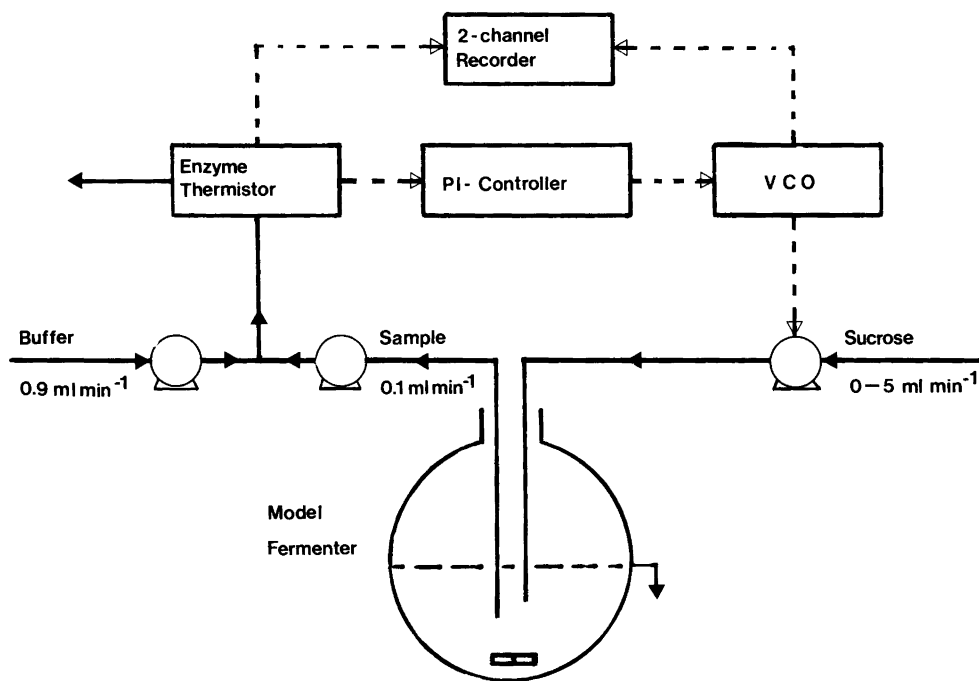


Fig. 1. Experimental arrangement. The sample and buffer flows were accomplished with a multichannel peristaltic pump with tubings and speed selected to give the flow rates indicated.

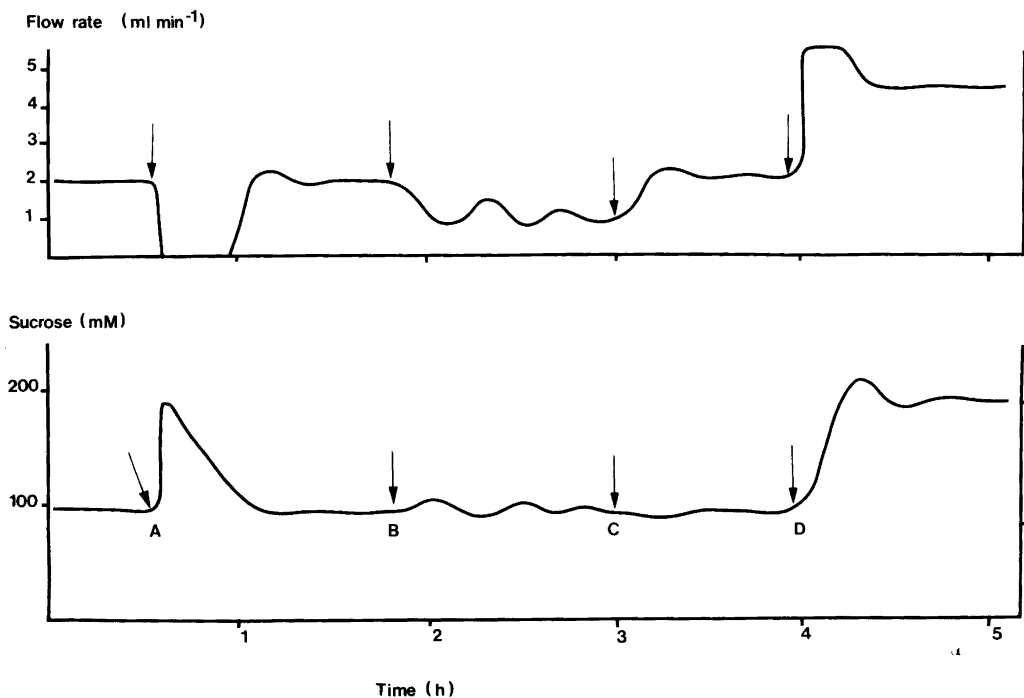


Fig. 2. Response of the control system to various disturbances. The upper curve shows the flow rate of the sucrose pump as a function of time. The lower curve is the thermogram of the enzyme thermistor and shows the sucrose concentration in the fermenter as a function of time. Four different types of disturbances are illustrated. In case A – C the sucrose concentration in the fermenter was set to 100 mM and in case D to 200 mM. (A) Additional sucrose was momentarily added to give a final concentration of 200 mM. (B) The sucrose concentration of the solution fed to the fermenter was changed from 250 mM to 500 mM and in C it was changed back again.

was packed with invertase (E.C. 3.2.1.26, β -D-fructofuranosidase) bound to pore glass coated with ZrO_2 (mean pore diameter 55 nm, 40–80 mesh; Corning Glass Works, U.S.A.). We applied 9 mg of invertase (Grade VI, from baker's yeast, 200 units/mg solid; Sigma Chemical Co., St. Louis, Mo., U.S.A.) to 0.9 mL of porous glass and coupling was accomplished as previously described.³ The enzyme thermistor response was found to be linear for sucrose concentrations in the range of 2–100 mM. A temperature response of 0.05 °C was obtained for 100 mM sucrose. No interference was observed for ethanol present in the samples in concentrations possible during the fermentation. Since the primary reaction is followed and no auxiliary enzymic steps are needed, the analysis is simple and is not influenced by other sugars, e.g., glucose. This is in contrast with methods for sucrose determination based on assay of glucose, which must involve a blank measurement of glu-

cose initially present in the sample.

The model fermenter consisted of a thermostated vessel equipped with a magnetic stirrer and level control. The experiments described here were carried out with 100 mL of spherical, 2 mm Calginate beads, of baker's yeast, containing 0.1 g yeast per ml of alginate beads.⁴ The beads were suspended in 0.1 M sodium phosphate + 0.05 M $CaCl_2$, pH 7.0, to give a total volume of 250 mL. The experimental arrangement is illustrated in Fig. 1. A peristaltic pump was used to continuously withdraw samples from the fermenter at a rate of 0.1 ml/min. Any gas bubbles present in the sample were separated through a Teflon membrane (Fluoropore filter, pore size 1 μ , Millipore Corp., Mass., U.S.A.). The sample flow was 10-fold diluted by mixing with buffer before introduction to the enzyme thermistor. Thus, sucrose concentrations in the fermenter of 20–1000 mM could be measured. A universal controller (Eurotherm, type 074,

Eurotherm Ltd., Broadmater, Sussex, U.K.) coupled to a voltage controlled oscillator was used to convert the enzyme thermistor signal to a frequency which could regulate the flow rate of the stepping-motor driven pump used for feeding the sucrose solution to the fermenter. Thus, for a given sucrose concentration of the solution added, a certain flow rate will be attained for which the sucrose concentration in the fermenter will be constant. If the latter concentration increases, the flow rate will be decreased and *vice versa*. The sensitivity of the control system to such variations can be changed by changing the proportional band of the controller and by changing the time constants of the integrating and derivating terms of the control function. Since the changes were comparatively slow, an integration time of 510 s was used. The derivating term was zero and the proportional band was 2% of the set sucrose concentration.

The response of the control system to various disturbances is shown in Fig. 2. It can be seen that the sucrose level rather quickly is brought back to the set value in all the illustrated cases. The parameters of the controller were set to give a rapidly decreasing oscillation.

Studies have also been made on fermentation in which glucose has been the substrate and the measurement carried out with a glucose oxidase/catalase thermistor. The model has furthermore been extended to include a continuous recovery system based on distillation for the ethanol formed.⁵

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