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Enzyme Thermistors for Process Monitoring and Control

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Abstract

In today's biotechnology there is an increasing demand for appropriate analytical systems for process control. At present the most widely used control systems are based on measurements of pH, pO_2, and pCO_2. Such systems do not allow the direct measurement of substrates and products. To overcome this drawback sensors such as enzyme thermistors and enzyme electrodes have been designed and their development into industrial useful sensors for monitoring and controlling is the subject of active research.

Index Entries: Enzyme thermistors, in process monitoring and control; thermistors, enzyme, in process monitoring and control; process monitoring and control; enzyme thermistors in; calorimetry, in process monitoring and control; immobilized enzymes, in process monitoring and control; glucose, enzyme thermistor for; sucrose, enzyme thermistor for

Experimental Procedure and Discussion

In the case of the enzyme thermistor (*1*), laboratory-scale control has been successfully demonstrated for an enzyme reactor model and for a fermentation in a continuous-stirred tank reactor. In the first study, an enzyme thermistor containing co-immobilized glucose oxidase and catalase was used to measure the amount of glucose in the outflow of the small bioreactor (50–250 mL) filled with

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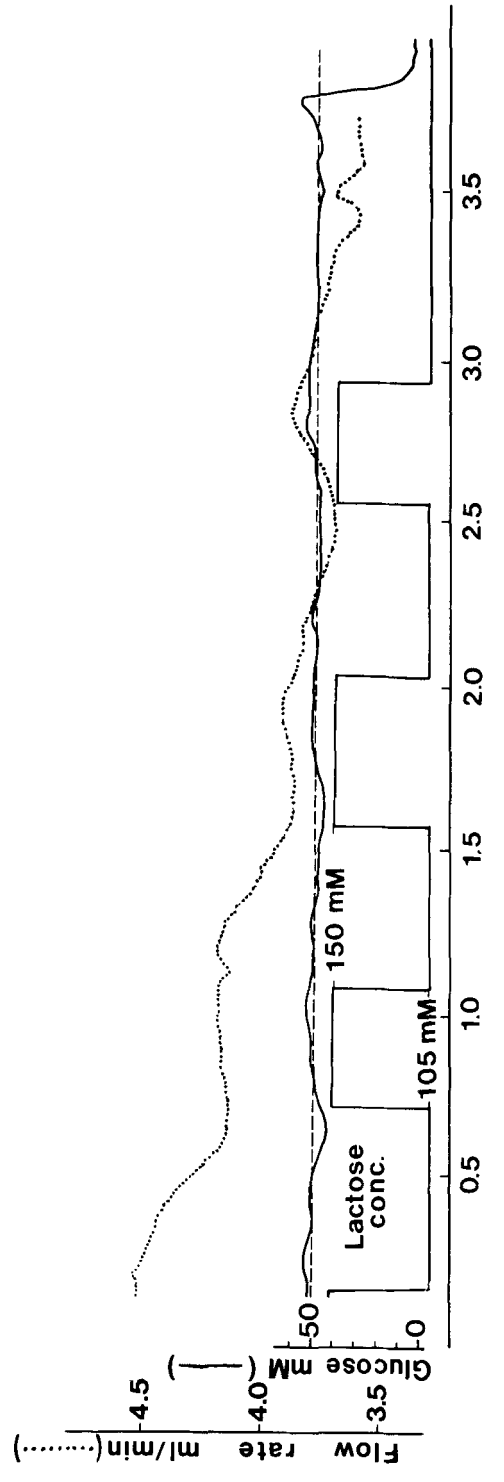

Fig. 1. Glucose concentration (——) and pump speed (·····) upon pumping whey through a 50 mL plug-flow reactor containing Sepharose-bound lactose. The glucose concentration was set to 50 m*M* (-----). Undiluted whey (50 m*M* in lactose) was introduced alternatingly with whey diluted to 70% as indicated in the diagram (2).

β-galactosidase covalently bound to Sepharose CL-4B (*2*). A fraction of the effluent from the reactor was diluted and pumped through the enzyme thermistor. The temperature signal was used via a controller to regulate the flow rate of the pump feeding lactose to the reactor. The product concentration (glucose) could be kept at a preset level despite variations in incoming lactose concentration, changing catalytic capacity, and so on. The performance of the system was investigated by introducing pure lactose solutions of slowly as well as rapidly changing concentrations. We also introduced whey, diluted or undiluted. These experiments were performed at 55°C, causing protein from the whey to precipitate in the lactose column, and thereby continuously decreasing its catalytic capacity. Changes in the operating conditions for the enzyme reactor are reflected in the pump speed, as can be seen from the example given in Fig. 1. The resulting glucose concentration was kept fairly constant throughout the experiment. Using whey as the substrate, the system could be operated for 3–4 h before total clogging of the enzyme reactor by whey proteins took place. The enzyme thermistor could, however, be used continuously over a longer period of time (1 day) before a recalibration was required.

The experimental arrangement shown in Fig. 2 was used for studies on the control of the substrate concentration in a continuous fermenter containing baker's yeast immobilized in alginate beads. The ethanol formed was continuously recovered by distillation while substrate was added with a pump controlled by the enzyme thermistor signal via a control unit as described in reference *3*. Glucose as

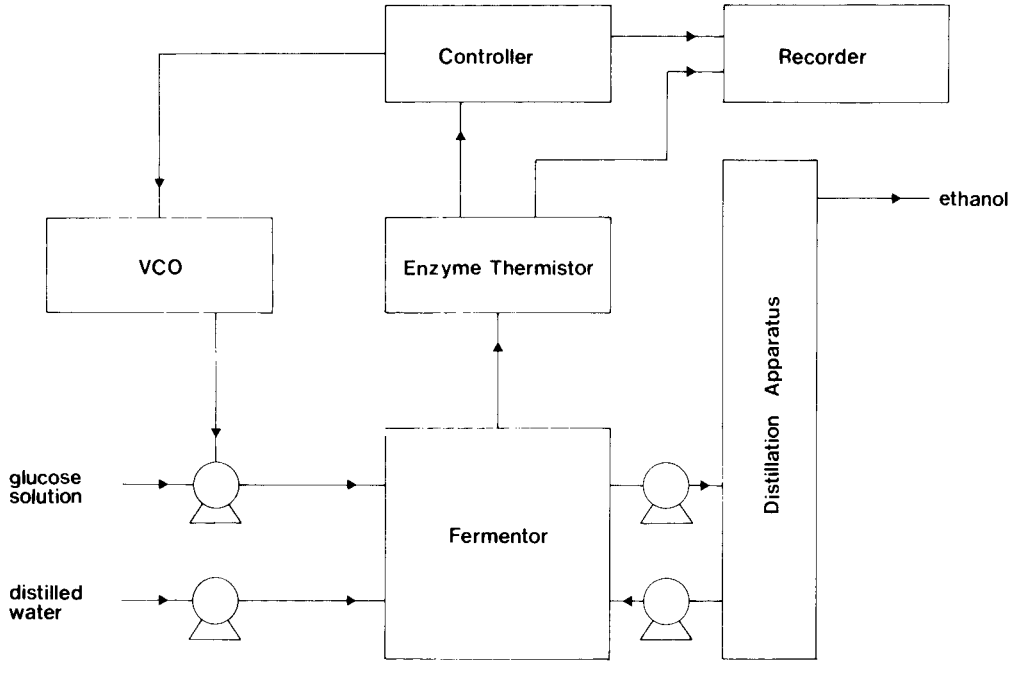

Fig. 2. Experimental arrangement for control of substrate concentration (sucrose) during continuous ethanol production by immobilized yeast. The enzyme thermistor was charged with immobilized invertase. The 250 mL fermenter contained 100 mL of alginate-yeast beads.

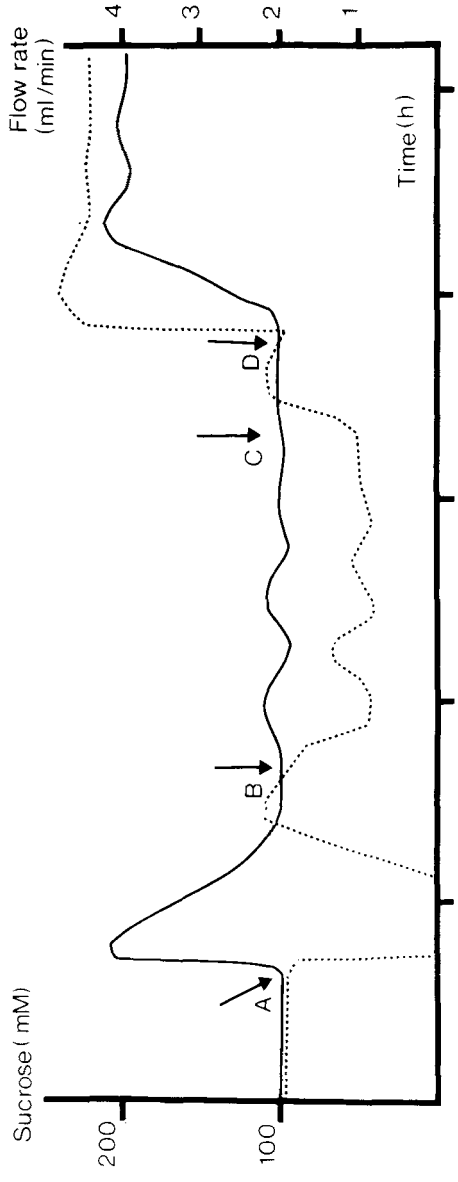

Fig. 3. Changes in the flow rate of the sucrose pump (----) at various disturbances. The sucrose concentration in the fermenter (———) was simultaneously registered. Four different types of disturbances were studied: In A–C the sucrose concentration in the fermenter was set to 100m*M* and in case D to 200 m*M*. (A) Additional sucrose was momentarily added to give a final concentration of 200 m*M*. (B) The sucrose concentration of the solution fed to the fermenter was changed from 250 to 500 m*M* and in C it was changed back again.

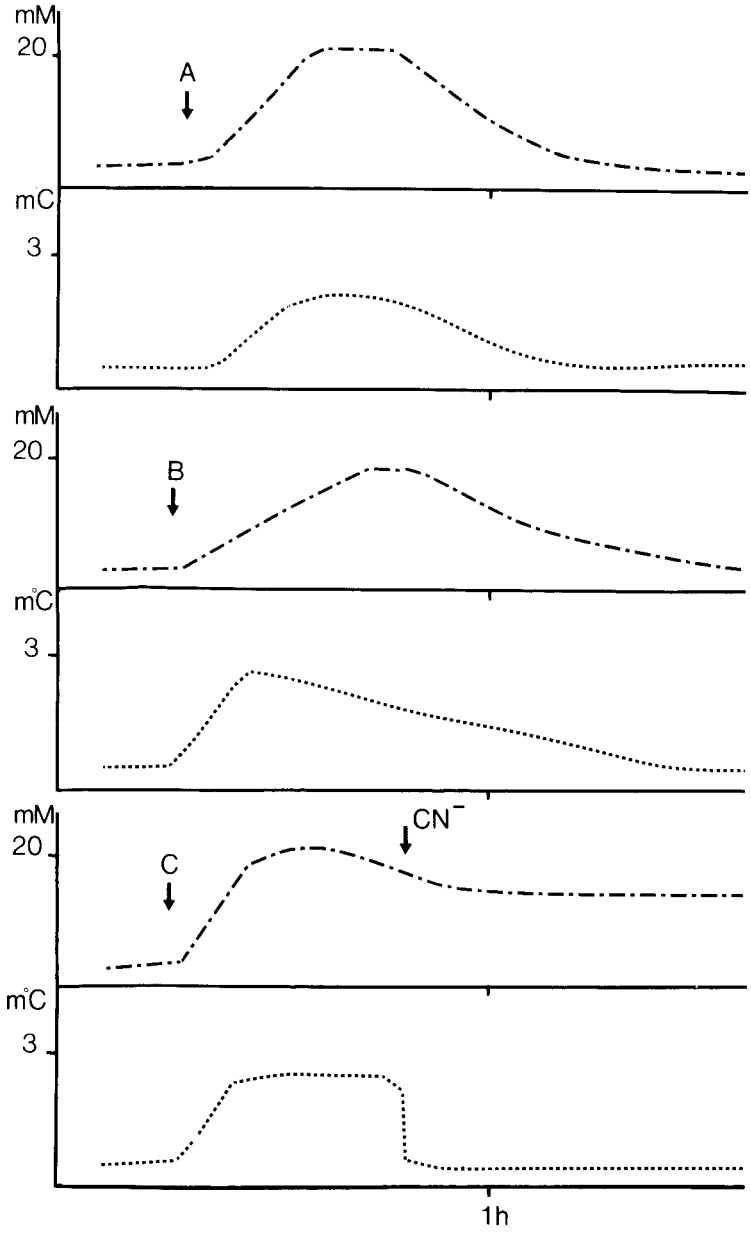

Fig. 4. Power–time curves (thermograms) registered for yeast (*Saccharomyces cerevisiae*). The heat production was monitored by continuously pumping (0.6 mL/min) broth from a 7-L fermenter into a modi fied enzyme thermistor unit. After temperature equilibration the yeast suspension passed through a 1 mL reaction coil. The glucose concentration was simulaneously monitored by a glucose oxidase/catalase thermistor. The results from three different studies are shown. The upper curve is the glucose concentration in the fermenter and the lower one is the thermogram. A, Glucose was added to a final concentration of 20 m*M*; B, sucrose was added to a final concentration of 34 m*M*; C , 40 g of glucose were added and at the point indicated by CN^-, cyanide was added to stop the metabolism.

well as sucrose were used as substrates. Glucose was measured by a glucose/catalase thermistor, sucrose by an invertase (E. C. 3.2.1.26) thermistor. Both systems performed well. The invertase thermistor is of particular interest since it allows determinations of sucrose over a wide concentration range (up to at least 100 m*M*) and has a remarkable operational stability. Figure 3 shows the variations in the substrate pump speed as a result of various disturbances.

Another interesting field of application for the enzyme thermistor unit is attained by replacing the enzyme column by a reaction coil through which a suspension of microorganisms from the fermenter is pumped. The heat evolution from the metabolism of the cells can thus be registered in a simple fashion. The potential of flow calorimeters for analyzing microbial processes (*4*) has long been recognized and it may be of interest to note that quite useful power–time curves (thermograms) of microbial activity can be also obtained with the comparatively simple enzyme thermistor unit. The results of some simple model experiments are shown in Fig. 4. The different metabolic responses to adding sucrose or glucose to a yeast suspension are illustrated as well as the drastic effect of adding cyanide to the same solution.

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