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A Glucose Electrode for Fermentation Control

SVEN-OLOF ENFORS

Department of Technical Microbiology, Chemical Center, PO Box 740, S-220 07 Lund, Sweden†

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Abstract

An oxygen-stabilized enzyme electrode was applied to monitor the glucose concentration in a fermentor during a batch culture of *Candida utilis*. The electrode contains an electrolysis circuit for generation of oxygen within the enzyme layer that keeps the oxygen activity in that layer at the same level as that of the surrounding broth. The electrolysis current is used as a measure of the glucose concentration in the broth.

The glucose analysis continued without major disturbances when the dissolved oxygen pressure gradually decreased during the fermentation and also when the broth was subjected to a sudden increase in dissolved oxygen tension. The electrode could also be used in an anaerobic broth. Then the reference electrode was replaced by a constant reference potential that simulated a reference oxygen activity.

Index Entries: Glucose electrode, for fermentation control; electrode, glucose, for fermentation control; fermentation control, glucose electrode for.

Introduction

The enzyme electrode technique has been utilized mainly in the clinical field. However, it should offer solutions to several critical problems in fermentation control where continuous monitoring of many substrates and products of biochemical reactions is a key problem (*1*). The main efforts in this direction have concerned penicillin electrodes (*2–5*).

†Present address: Dept. of Biochemistry and Biotechnology, Royal Institute of Technology, S-100 44 Stockholm, Sweden.

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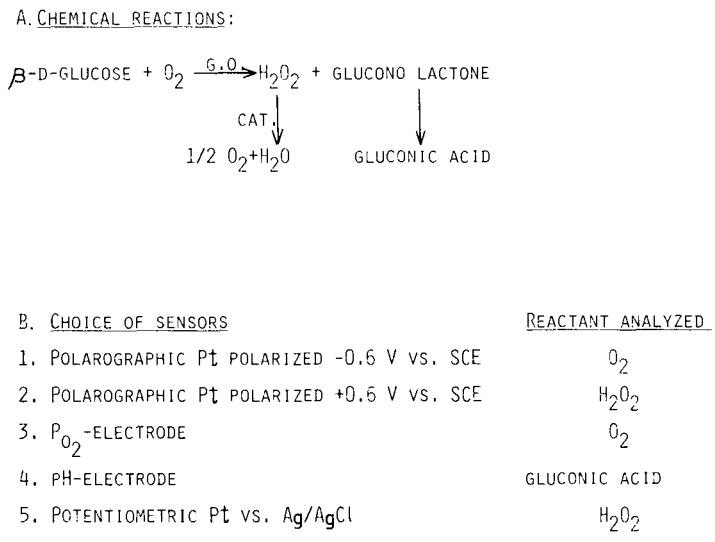

Fig. 1. Biochemical (A) and electrochemical (B) basis of glucose electrodes. The enzyme must not contain catalase if sensor No. 2 is used.

One of the most interesting enzyme electrodes for fermentation control would be a glucose electrode. Several designs of glucose electrodes have been presented. A summary of the biochemical and electrochemical basis of different glucose electrodes is given in Fig. 1. For reviews, see refs *6* and *7*. However, the glucose electrodes hitherto described suffer from three limitations with respect to application in fermentation control: (1) the demand for a high oxygen content in the sample; (2) the low upper concentration limit for glucose analysis; and (3) the lack of any sterilization possibility.

One solution to the oxygen demand problem was presented by Romette and coworkers (*8*), who made a glucose electrode with an enzyme membrane in which oxygen was dissolved to a very high level prior to the analysis. This oxygen reservoir then furnished the reaction, also in oxygen free samples, with sufficient oxygen for analysis according to the kinetic method. However, this method, though suitable to semicontinuous analysis, would not be applicable to analyses *in situ* in a fermentation process.

This paper describes an oxygen-stabilized enzyme electrode (*9*) applied to glucose analysis in a fermentation broth during a process with varying dissolved oxygen tensions and under anaerobic conditions.

Materials and Methods

The Oxygen-Stabilized Glucose Electrode

The principle of the electrode is shown in Fig. 2. It is based on the galvanic oxygen electrode of the Johnson-Borokowski-Engblom type (*10*). On the membrane is attached a Pt screen (ϕ, 8 mm; mesh size 24) with immobilized glucose oxidase and

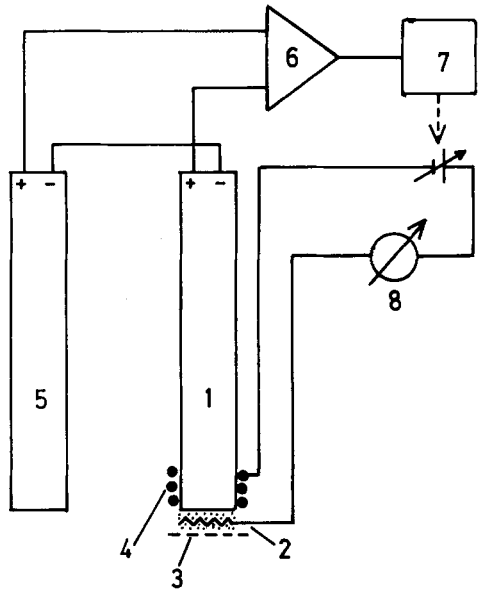

Fig. 2. Principle of the oxygen-stabilized glucose electrode: 1, oxygen electrode; 2, Pt screen with immobilized glucose oxidase and catalase; 3, dialysis membrane; 4, Pt coil wired around the electrode body; 5, reference oxygen electrode; 6, differential amplifier; 7, Pl-controller; 8, μA-meter.

catalase. A dialysis membrane (porosity, 25 Å) separates the enzyme from the broth. The Pt screen is connected as the anode in an electrolysis circuit, the cathode of which is a Pt wire (ϕ, 0.75 mm; length, 400 mm) wired around the electrode body as close to the anode as possible. For a reference electrode, a similar oxygen electrode without electrolysis electrodes and enzymes is used.

Both electrodes are calibrated to give equal signal outputs in a glucose-free medium. The differential signal output between the glucose electrode and the reference electrode is used to control the electrolysis current. When glucose diffuses into the enzyme, the enzymatic reaction will reduce the oxygen activity in the enzyme electrode, and this will cause a differential signal output that starts the generation of oxygen by electrolysis at the anode. In this way the oxygen activity in the enzyme electrode will be kept constant at the same level as that of the broth. The higher the glucose concentration in the broth, the higher will be the oxygen consumption rate of the enzyme. As long as the oxygen activity is kept stabilized by electrolytic generation of oxygen, the electrolysis current will be a measure of the oxygen consumption rate provided that no electrochemical side reactions occur.

Preparation of the Enzyme Anode

A Pt screen was washed with conc. nitric acid, acetone, and distilled water, respectively. A 20–mg quantity of glucose oxidase (β–D-glucose: oxygen oxidoreductase, EC 1.1.3.4) from *Aspergillus niger* (Koch-Light Lab. Ltd., England) containing 1.35 GO units/mg (approximately 0.6 U/mg) and 1 mg cata-

lase (hydrogen peroxide, hydrogen peroxide oxidoreductase, EC 1.11.1.6) from beef liver (United States Bio-chemical Corp., Ohio, USA) containing 5755 U/mg, were dissolved together with bovine albumin (BDH Chemicals Ltd., England) in 0.25mL of 25m*M* phosphate buffer at pH 7.0.A 0.1 mL volume of a 2.5% solution of glutaraldehyde (Merck, W. Germany) was added and the Pt screen was wetted with the mixture and then placed in a refrigerator for polymerization over night.

Fermentation

Candida utilis was grown at 30°C in 800 mL broth with the composition, (g/L): Glucose, 2; $(NH_4)_2SO_4$, 2; yeast extract, 1; Na_2HPO_4, 0.22; KH_2PO_4, 0.66; $MgSO_4$, 0.5; Na citrate, 0.005; and $FeCl_3 \cdot 6H_2O$, 0.0005. The fermentation was performed in a 1-L fermentor (Chemoferm AB, Sweden) at the aeration rate 0.4 L/min and a stirrer speed of 700 rpm. In order to increase the dissolved oxygen tension these values were increased to 0.8 L/min and 1000 rpm, respectively. The pH was kept constant at 6.0 by titration with 0.5*M* NaOH. The amount of dosed NaOH solution was monitored with a Dose Monitor (ServoChem AB, Sweden), which yielded a curve that is proportional to the biomass curve.

Measurements under anaerobic conditions were performed with a glucose-free and uninoculated broth under the conditions mentioned above. The reference electrode was then replaced by a dc voltage source adjusted to a potential corresponding to the reference electrode potential in air saturated broth. In this way the glucose electrode was forced to keep a proper internal oxygen activity. The calibration curve was then produced by known additions of a concentrated glucose solution.

Control analyses of glucose were performed in samples withdrawn from the fermentor. After sterile filtration, the filtrate was analyzed with a Gluco-quant kit (Boehringer Mannheim GmbH, W. Germany).

Results and Discussion

Figure 3 gives data from a batch culture of *Candida utilis*. The glucose electrode signal (i.e., the electrolysis current) describes the expected course, i.e., an inversion of the growth curve (visualized by the dose monitor curve). Control analyses showed a good correlation between the electrode signal and glucose concentration, as shown in Fig. 4. During the last hour (5th–6th h) of the process, when the rate of glucose consumption was highest, there was a too-high electrode signal. This is also evident from the high electrode signal when the growth ceased. However, this was found to be caused by a non-optimal adjustment of the Pl-controller. At time "4 hours" in the process, the oxygen transfer was increased in order to substantially change the dissolved oxygen tension. Figure 3 shows that the electrode did not respond markedly to this disturbance. Thus, the oxygen-stabilized glucose electrode worked essentially independent of the surrounding dissolved oxygen tension.

One very important conclusion of this experiment is that in this typical fermentation broth there occurred no errors caused by unwanted electrochemical side reac-

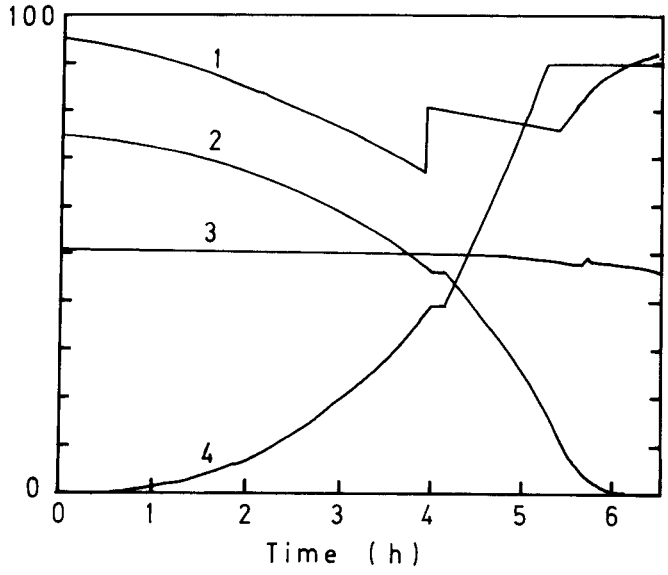

Fig. 3. Recorder plots from a batch culture of *Candida utilis* in mineral salts medium, initially supplemented with 2 g/L glucose: 1, dissolved oxygen tension of the broth (0–100% air saturation value); 2, electrolysis current (0–200 μA); 3, differential dissolved oxygen tension (−25– +25% air sat.); 4, growth curve (obtained by means of a Dose Monitor, arb.scale).

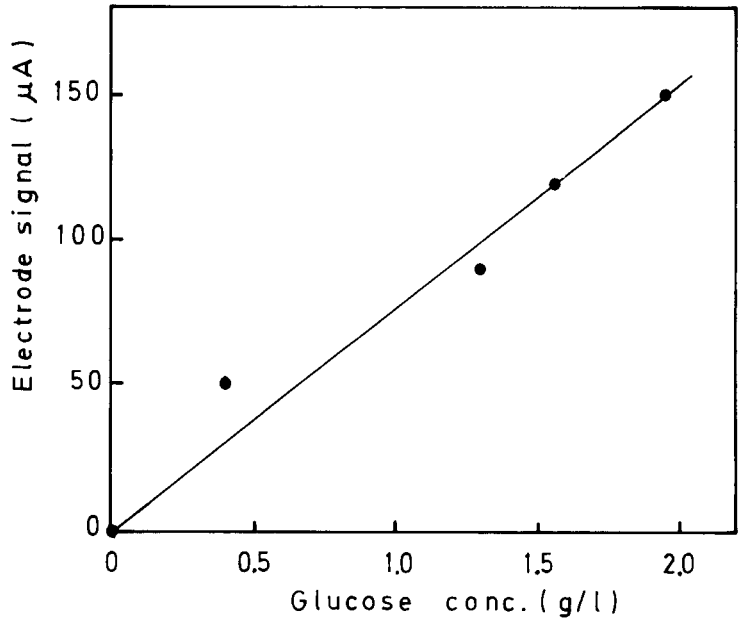

Fig. 4. Correlation between the glucose electrode signal and the glucose concentration of the *C. utilis* broth according to control analyses.

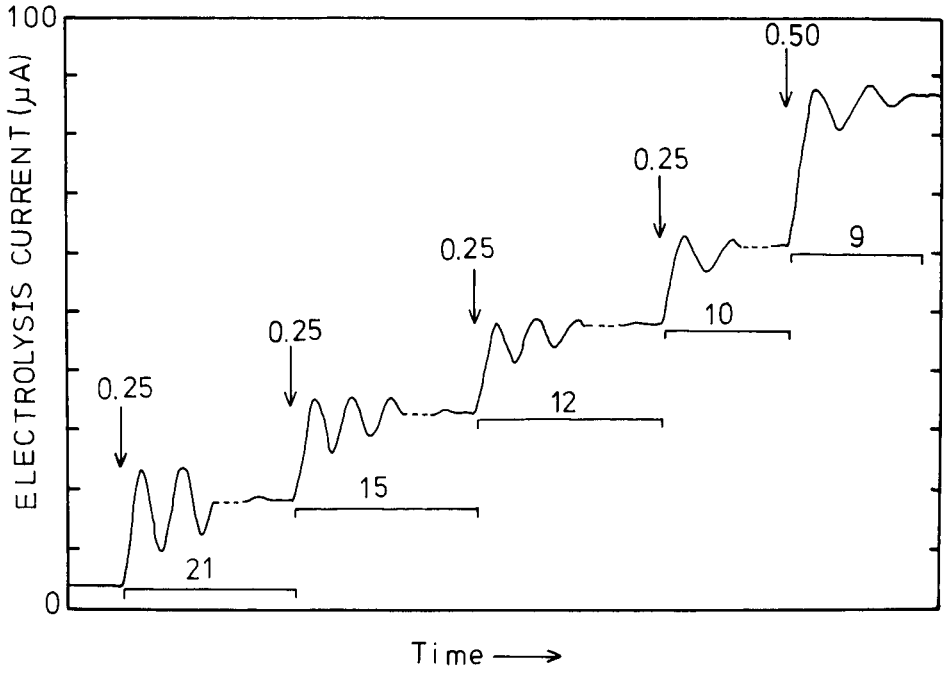

Fig. 5. Response of the glucose electrode to additions of glucose in oxygen free broth. Figures and arrows indicate the additions (g/L). Bars and figures indicate the time required to stabilize the electrode after each addition (min).

tions, which could give a back-ground current in the electrolysis circuit without corresponding oxygen generation.

In order to extend the measurements to completely anaerobic media, the reference electrode was replaced by a constant reference potential, as described above. Figure 5 shows that a basic electrolysis current of 4 μA was needed to generate the proper oxygen activity in the enzyme when no glucose was present. Glucose additions then increased the current to levels proportional to the glucose concentration. The long response times were caused by non-optimal adjustment of the PI controller, and it ought to be possible to substantially improve this point. However, the experiment shows that the glucose electrode can work without oxygen transfer from the sample to the enzyme. This will also enable the extension of the measuring range to higher concentration.

In summary, these experiments demonstrate that the oxygen-stabilized enzyme electrode, in the glucose version, works in a typical fermentation broth with varying dissolved oxygen tensions and under complete lack of oxygen.

Acknowledgments

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