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# Measurement system of low glucose concentration during the cultivation of yeast cells

Tomokazu KISHIMOTO*, Seiichi HARA**, Masafumi MURAJI***
Hiroaki TSUJIMOTO****, Masayuki AZUMA*****, Hiroshi OOSHIMA******

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## Synopsis

A yeast cell changes an active state in accordance with glucose concentration in a culture medium. Below a certain critical glucose concentration under aerobic conditions, the yeast respires. Exceeding its value, the yeast changes an active state to fermentation. The aim of our study is to maintain the state of respiration and fermentation of yeast artificially. And so, a glucose sensor was needed to satisfy with respiration condition. In this study, we tried to construct the glucose sensor which was to measure glucose concentrations in very low region for a long time and to maintain quasi realtime measurement. The sensor was constructed using the phenomena of light emission by luminol, we evaluated the sensitivity, stability and reliability of it. The sensor was robust against outer disturbances, and had an influence by flow rate of solution, and dialysis rate. A detailed explanation of aerobic conditions and of reaction principle of the constructed glucose sensor will be presented here. And then, some basic characteristics of the glucose sensor will be shown here as well.

## Introduction

In the last few decades, several articles have been devoted to the study of transient behaviour of yeast on the continuous measurement of glucose. Those refer to the yeast behaviour at each transient phase of the respiration, aerobic alcohol fermentation and fermentation in the addition of glucose. The purpose of our studies here is not to observe such yeast behaviours, but to distinguish the respiration from the fermentation more clearly. A yeast cell (Saccharomyces cerevisiae) performs respiration or fermentation according to the conditions of cultivation. It is well-known that it respires in aerobic conditions (chemical formula (1)) and ferments in anaerobic conditions (chemical formula (2)). That, however, depends on environmental factors to control the branching point of respiration and fermentation. Of environmental factors, the dissolved oxygen

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* Master student, Department of Physical Electronics and Information
** Master student, Department of Physical Electronics and Information
*** Lecturer, Department of Physical Electronics and Information
**** Professor, Department of Physical Electronics and Information
***** Associate Professor, Department of Applied and Bioapplied Chemistry
****** Professor, Department of Applied and Bioapplied Chemistry

concentration and the glucose concentration are the most important parameters [1]. Cornelis Verduyn et al. refer to the fact that it respires when the glucose concentration of a culture medium is below its threshold value of $8x10^{-4}$ $\mu$M in addition to the aerobic cultures, and that when exceeding its value, the yeast behaviour shifts to the manifestation of fermentation either in the aerobic or anaerobic conditions. And so, the sensor with high sensitivity was required since the glucose concentration to be measured was below the detection limit of other methods such as commercial glucose sensors and glucose kits for the purpose of satisfying with conditions of respiration. Furthermore, glucose sensor was needed that the measured data could be mechanically stable and reliable, and that it was possible to measure continuously, for a long time.

$$C_6H_{12}O_6+6O_2 \longrightarrow 6CO_2 + 6H_2O \quad (1)$$

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

In this study, we constructed the glucose measurement system during the yeast cultivation in which low glucose concentration measurement is possible.

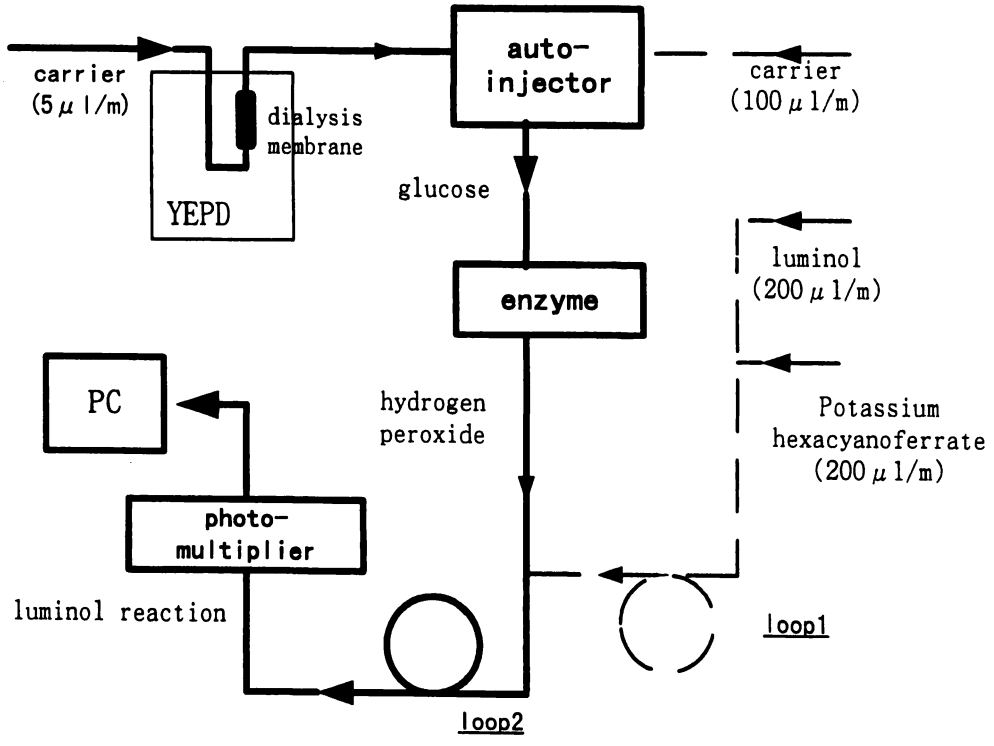


Fig1. The flow chart of glucose measurement system

We made use of luminol reaction to acquire an accurate concentration of glucose. The reaction was induced by consolidating three solutions; phosphate buffer [PH6.0] used as carrier, potassium ferricyanide [PH9.0] used as catalysis and luminol [PH9.0], into one at the converging point of three tubes to run the respective solusions. The phosphate buffer contained sodium azide 1mM to destroy catalase reacting with the produced hydrogen peroxide chemically (catalatic reaction).

First of all, the hydrogen peroxide corresponding to the concentration of dialyzed glucose was produced through the glucoseoxidaze of immobilized enzyme as shown in the Fig1. Then, luminol and Potassium ferricyanide are added to the produced hydrogen peroxide and the luminol reaction was induced, and finally the amount of luminescence (in proportion to the amount of hydrogen peroxide) is measured and then recorded continuously in PC after converted into the output voltage using a photomultiplier. The dialysis membrane is a sampling point of glucose, which was immersed in YEPD medium and used so as to avoid making an intrusion of yeast into the autoinjector. Its flow chart is shown in Fig1. Two loops were used to diffuse each solution in the tube.

The design of glucose sensor was paid much attention to the following two points. One is that the output voltage is mechanically stable and reliable. For its purpose, the photomultiplier was introduced that coverts incident light into photoelectons for the purpose of preventing voltage rises by the drift and not-negligible noise from other electric appliances so as to make the measurement for a long stretch of time possible. Another is that quasi realtime-monitering measurement is feasible. For its purpose, the autoinjector is carried out once in 3 minutes, the lag time of measurement from injection to the record in PC is set in about one and a half minute.

Their experiments were performed under the following conditions: (a) the glucose was dissolved in distilled water, (b) the glucose was dissolved in a culture medium of YEPD (1.0% yeast extract, 2.0%peptone, PH5.0), all measurements were made in 100ml volume of a glass beaker.

## Results & Discussion

We show the basic characteristics of the constructed glucose sensor. The relationship between the glucose concentration and the output voltage of the photomultiplier is shown in Fig. 2 (a) and (b).

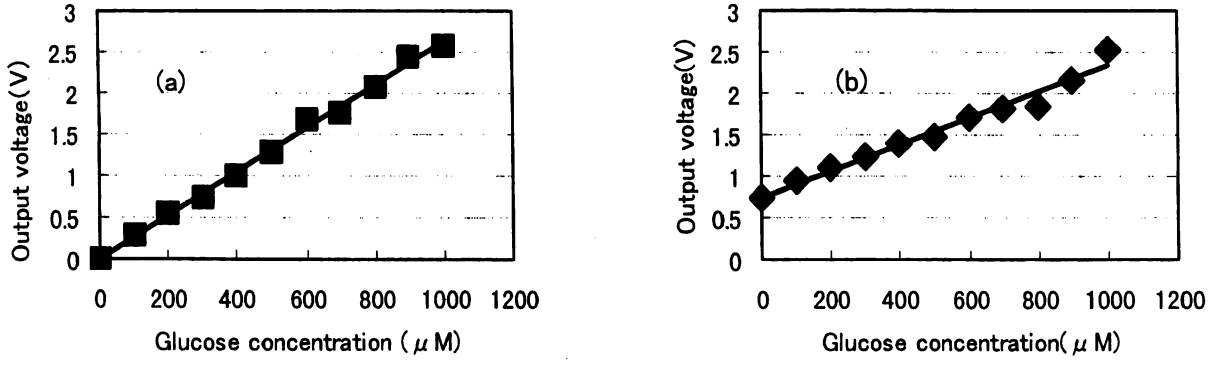


Fig.2. Calibration of the glucose sensor by the addition of a standard glucose to the distilled water (a) or to the YEPD medium (b) via a pipet.

The output voltage of the constructed sensor gave an almost linear response to glucose additions up to 1000 μ M every 100 μ M glucose. The linear approximation was applied to each data of figure. It is notable that Fig2(b) has the output voltage of about 0.7V at no glucose. Why was bottomed up at initial glucose concentration in Fig2(b) can be explained by that glucose-containing Yeast Extract is dissolved as one component of YEPD medium.

Fig3 shows the characteristics of dialysis rate for additions up of glucose. The aim is to acquire the calibration of time course of the dialysis rate and to evaluate the influence of the varied glucose concentration on the performance of the dialysis membrane. The dialysis rate was defined as the ratio of glucose in dialyzing between membranes, depending on the concentration gradient. Eventually it was the value dividing the output voltage obtained by that of the standard glucose at respective glucose concentrations. Each of triangle mark is represented as the average value of 15 measured data, the average value and the perpendicular line on the marks denote the normalized, relative dispersion value on the standard glucose concentration 100 μ M respectively. Judging from the outline of measured data, the approximation line was just drawn smoothly. This result produced an effect contrary to our intension. however, it is noteworthy that the relative ratio is getting lager as addition up of glucose. Generally, the dialysis rate increased up exponentially depending on concentration gradient. However, it drew logarithmic curve approximately. For its reason, it is possible to build up one hypothese that the dialysis of glucose between membranes may be saturated due to the adhesion of the protein contained in YEPD medium onto membrane. As shown in Fig3, it can be found that the dispersion value increases as the glucose concentration does, it was, however, kept low around the measured value 400 μ M which can satisfy with the conditions of respiration. The flow rate of the carrier 100 μ l/m was determined as set value because the largest output voltage can be obtained (data not shown).

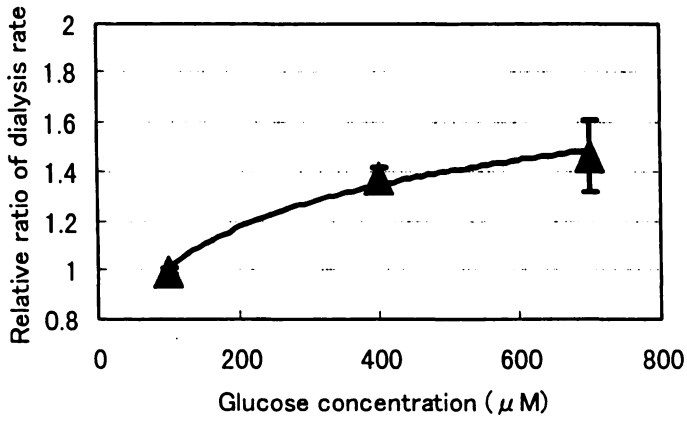


Fig3. The normalized relative ratio of dialysis rate

The configuration of the sensor could make the adaptation to different and innovative environments possible, that is, give a much boarder range of application for the measurement of solutions. For instance, if substituting three solutions used to induce luminol reaction for other ones to do light emission by chemical reaction, or if making use of appropriate combination of the enzyme and the substrate for its enzyme. It wouldn't be an exaggeration to say that the sensor holds the potential for the application measuring other concentration of solution. For subsequent development, it is necessary to find out the solutions and calibration curves for compensations of the measured output voltage because it is influenced by the adhesion of yeast cell itself onto the dialysis membrane, or by the deposition of proteins of intermediate products onto one which yeast cells will produce in the process of respiratory chain throughout TCA cycle.

## conclusion

As mentioned in the synopsis before, the purpose of the experiment here was to establish an active state of respiraton of yeast cells. For its purpose, we constructed the measurement system to measure the low glucose concentraion for respiration of yeast. Considering the figures illustrated on this article, we can say that the sensor had a linear property for addition up of glucose, appropriate calibration curves of dialysis rate as well. It can be concluded that the results have been so far reflecting the objective of sensor construction, though there would be left some problems to find out solutions for subsequent development.

## References

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