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Application of microthermometry to measurement of microbial activity and inactivation process by inhibitor

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Abstract

A rapid and simple technique (microthermometry) was developed for the measurement of microbe's metabolic activities and its inactivation process by an inhibitor. To analyze the results and to determine the parameters for the estimation of the activity and inactivation degree, a simple model was proposed. Yeast cells (*Saccharomyces cerevisiae*) were used as a microbe. A differential method using two bead-type thermistors as reference and measuring probes was employed for the detection of a temperature change caused by the heat of metabolism of added carbon source (glucose) by the yeast. Experiments were conducted in a 30 °C water bath under non-growth conditions (without nitrogen source). The simple thermal response model was applied to obtain two characteristic parameters for the estimation of the yeast activities, i.e., a metabolic heat production rate, ΔQ, and a metabolic heat inhibition rate, ΔK. The proposed model was well in agreement with the experimental results, and the curve fitting gave ΔQ or ΔK. In the case of the addition of glucose to yeast as a carbon source, ΔQ was proportional to the number of live cell (CFU). The slope of CFU versus ΔQ for yeast in exponential phase was larger by about two-fold than that in the stationary phase. In the case of the addition of gultaraldehyde as an inhibitor to the above system, the logarithmic value of ΔK increased with increasing the concentration of gultaraldehyde within the range of 0.02–6.2 wt.%. It was suggested that the two model parameters, ΔQ and ΔK, can be regarded as characteristics to estimate the activities of bacteria and the degree of their inactivation by the inhibitor.

Keywords: Yeast; Glucose; Microbial activity; Metabolic heat; Thermistor; Bioprocess monitoring

1. Introduction

Recently an assessment of hazardous substances in aqueous environments, which is known as bio-monitoring and microbiotesting, was studied [1]. In such techniques, a rapid estimation method of microbe's metabolic activity and inactivation process by an inhibitor is required. However, most of the techniques were somewhat time-consuming or tedious and were required expensive apparatus or reagents, because they are based on enzymatic or bioassay methods.

Cooney et al. [2] attempted to apply calorimetric or thermometric method to biochemical and bioengineering fields. They reported a correlation between heat evolution and oxygen consumption of microbes in a fermentor using a thermistor. In recent two decades, many successful attempts using thermistor devices were reported; e.g., enzyme thermistor [3–7], microbe thermistor [8], enzymatic kinetics [9–11] and so on. Furthermore, many studies from the viewpoint of thermometric aspects about biological and physical properties of microbes have appeared and they often used micro calorimeter [12–19].

Because the thermometric methods using thermistor devices are based on a very general detection principle, it is reasonable to consider that the methods make it possible to develop a compact apparatus and on-line measurement of activity of microbes. However, applications of activity and inhibition estimation by micro-thermometry to microbes have not appeared in literature and have not been developed yet.

In this study, the small change in temperature due to the emission of metabolic heat by microbes will be measured by a differential detection method to characterize the microbial activity and the inactivation process of microbes by an inhibitor. The measurement will be carried out by a system consisting of two thermistors to eliminate the effect due to any other undesirable sources of heat. The obtained thermal data will be analyzed by a simple kinetic model derived from heat balance and will be determined two parameters to estimate

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Nomenclature

A	interfacial area (cm^2)
c_p	specific heat of suspension (J/(g °C))
ΔK	the metabolic inhibition rate estimated by Eq. (7) (J/s)
ΔQ	the metabolic heat production rate estimated by Eq. (3) (J/s)
t	time (s)
ΔT	differential of temperature between T_m and T_r (°C)
T_m	temperature of the yeast suspension in the measuring tube (°C)
T_r	temperature of the yeast suspension in the reference tube (°C)
U	overall heat transfer coefficient (J/(°C cm^2 s))
w	weight of yeast suspension (g)

Greek symbol

τ	time constant (s)

microbial activities and the degree of their inactivation by an inhibitor.

2. Description of unsteady heat balance model

The heat balance of the present micro-thermometry system in unsteady state gives the following equation:

$$wc_p\frac{d}{dt}(T_m - T_r) = (Q_m - Q_r) - UA(T_m - T_r), \quad (1)$$

where T and Q represent the temperature and the production rate of heat by yeast cells, respectively, and the subscripts m and r denote the measuring and the referring tubes, respectively. w and c_p represent the weight and the specific heat of yeast suspension, respectively. A and U denote the heat transfer area and the overall heat transfer coefficient between the water bath and the yeast suspension, respectively. Substitution of ΔT for $T_m - T_r$ and ΔQ for $Q_m - Q_r$, respectively, and rearrangement of Eq. (1) lead to the following equation:

$$\frac{wc_p}{UA}\frac{d}{dt}\Delta T - \Delta T = \frac{\Delta Q}{UA}. \quad (2)$$

Solving the above differential equation with the initial condition that $\Delta T = 0$ at $t = 0$, the net change in temperature due to the metabolism of yeast cell, ΔT, can be finally obtained as

$$\Delta T = \Delta T_s(1 - e^{-t/\tau}), \quad (3)$$

and

$$\Delta T_s \equiv \frac{\tau}{wc_p}\Delta Q. \quad (4)$$

where the time constant, τ, in Eqs. (3) and (4) is defined as

$$\tau \equiv \frac{wc_p}{AU}. \quad (5)$$

As is well known, Eq. (3) is a response of temperature in thermometric system for a stepwise change in the metabolic heat production rate, ΔQ, of yeast cells by injection of glucose.On the other hand, an estimating equation of cell inactivation rate, ΔK, is derived from the following equation as the same manner in case of ΔQ,

$$-wc_p\frac{d}{dt}\Delta T = \Delta K + UA\Delta T, \quad (6)$$

ΔT can also be given as the following equation by solving Eq. (6) with the initial condition of $\Delta T = 0$ at $t = 0$,

$$\Delta T = -\frac{\tau}{wc_p}\Delta K(1 - e^{t/\tau}), \quad (7)$$

Eq. (7) is also the response of temperature in thermometric system for a stepwise change in ΔK of yeast cells by injection of an inhibitor.

Eqs. (3) and (7) will be verified with the experiments and two characteristic parameters, ΔQ and ΔK, will be determined by fitting to the experimental results.

3. Experiments

3.1. Microbial strain

Saccharomyces cerevisiae (IFO No. 2043), yeast cells, was used as a model microbe. The cells were inoculated with nutrient-rich broth consisting of 20 g glucose, 2.0 g KH_2PO_4, 1.0 g $MgSO_4{\cdot}7H_2O$, 5.0 g yeast extract and 5.0 g $(NH_4)_2SO_4$ per liter at 30 °C for 24 h and were stored in the broth containing 15% glycerin at −40 °C. All chemicals were analytical grade and used without further purification.

3.2. Preparation of yeast suspension

The yeast cells were grown in 400 mL nutrient-rich broth for 10 h (exponential phase) or 20 h (stationary phase) at 30 °C. After that, they were harvested by centrifugation and washed two times with distilled water to prepare desirable concentrations of yeast suspensions. In all runs, 2 mL of the suspension was used as a sample to measure the change in temperature due to metabolic heat. The number of live cells was determined by colony counting on agar plate.

3.3. Experimental set-up

Measurement of the change in temperature due to metabolic heat was conducted in a 30 °C water bath. Two test tubes, a measuring and a reference tube, were placed in the water bath. A thermistor used in this study was a bead type thermistor (ET-104, 100 kΩ at 25 °C, B constant 4132 K, Ishizuka Electronics Corporation). A differential method using two thermistors was employed in this study to reduce any non-specific signals. Two thermistors were incorporated into individual legs of Wheatstone bridge circuit. Voltage signal from the circuit was monitored by a voltmeter (VOAC 7411, Iwatsu Electronics Co. Ltd.) and was recorded by a personal computer (NEC PC-9801