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# DESIGN AND TESTING OF A CALORIMETER FOR BIOLOGICAL USES

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ABSTRACT

A rotatory microcalorimeter of the conduction type has been designed for the study of microbial metabolic processes under both aerobic and anaerobic conditions. The instrument can be performed in either batch mode or flow mode by changing the calorimeter vessels and the tube connections. The sensitivity and the time constant were determined by electrical calibrations. The heat sensitivity was 0.12 mV/mW with both yeast and other fungi. Because of the sufficient aeration and agitation, the calorimeter is available for studies on the fungi growth experiments of biotechnical interest.

## INTRODUCTION

Calorimetric investigation on living cells represent a vast experimental area, however, most of the interesting studies are restricted so far to microbial anaerobic processes. This is because the calorimetric operation of aerobic processes with heavy or large microbe is technically more difficult than bacterial unaerobic processes.

For system with a very high oxygen consumption, such as yeast and other fungi growth processes of biotechnical interest, there appears to be no suitable calorimeter available as yet. For these purpose we build a rotatory microcalorimeter of the conduction type for continuous studies of aerobic microbial processes by batch operation (ref.1). Depending on the nature of the problem under study, both the batch and the flow calorimeter have proved to be useful in different areas of biological analysis, e.g. in biotechnology, ecology and in clinical work. A large amount of calorimetric methodological work of this nature has been conducted during the last some years, see e.g. (refs.2,3).

Generally the flow calorimeter has various advantages compared with the batch calorimeter. The calorimetric experiment can be made exceedingly simple and the procedure is well suited for sample analysis. However, there are some systems which are not well suited for measurements with the flow calorimeter, e.g. microbial measurements in food systems or studies with certain cells which tend to adhere to the walls of the flow lines and to the flow vessel. To extend the use of our calorimeter system for theses areas, a new calorimeter has been

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designed. The underlying idea has been that it should be possible to change an instrument only slightly in order to obtain another one with a different function. Design and testing of a calorimeter to meet these conditions will be described in this paper.

## EXPERIMENT

### Calorimeter

The calorimeter used in this study is a modified version of the rotation calorimeter MODEL MPC-III commercially available from Tokyo Riko. A special feature of the calorimeter with heat excahngers is available to use for either flow mode or batch mode by changing the vessels and the tube connections.

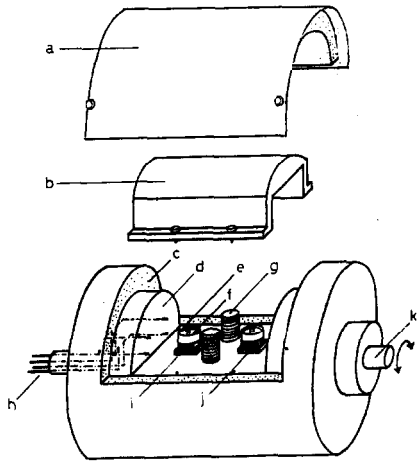


Fig. 1 (left). Sketch of the rotatory microcalorimeter.
(a),styrofoam insulation lid; (b),alminum lid of heat sink; (c),styrofoam thermal insulation; (d),heat sink; (e),vessel holder; (f),vessel; (g),heat exchanger; (h),inlet and outlet tubes; (i),heater; (j),thermomodule; (k),rotation axis.

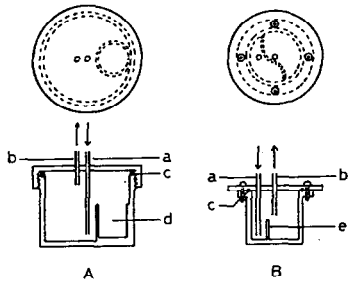


Fig. 2 (right). Calorimeter vessels.
(A) Batch vessel. (B) Flow vessel. (a),inlet tube; (b),outlet tube; (c),O-ring; (d),small compartment; (e),baffle plate.

A schematic view of the calorimeter is shown in Fig. 1. An aluminum block serving as a heat sink contains two identical reaction vessels, one for active and the other for reference uses. The temperature of the calorimeter is regulated within ± 0.005°C by a thermostated air bath. Thermopile plates situated between the vessels and heat sink are used for temperature indication with

a temperature sensitivity of 29 mV/°C and serve also a path through which heat passes from the reaction vessel to the sink or vice versa. The flow line is consist of two heat exchngers (wound with 2 m of tefuron tube) set on the heat sink and four tube connectors made of stainless steel. They are connected with the active vessel and the culture fluid and air are supplied from the outside of the calorimeter into the vessel through the flow line. The calorimeter can be rotated 90° clockwise and anticlockwise alternately up to 120 r.p.m..

Culture vessels

The batch and flow vessels are shown in Fig. 2. The batch vessel (A) made of stainless steel is a cylindrical flask with a diameter of 30 mm and a hight of 20 mm. The vessel contains a small compartment with the volume of 1 ml which separates the inoculum and the medium prior to operation. The maximum content of liquid is 5 ml and two pipes fastended to the lid are used for aeration. The flow vessel is also a cylindrical flask with a diameter of 15 mm and a hight of 14 mm. Liquid and air flow through the vessel by two tubes fastend to the lid. The retention volume is 1.4 ml and a baffle plate is placed in the vessel to improve the efficiency of the agitation.

Culture assembly

Both arrangements for the batch system and the flow system are shown in Fig. 3. In the batch system (A), cells are cultured in the batch vessel to which air is supplied quantitatively by a peristaltic pump via a strilizing filter. An additional heat exchanger placed in the air bath is used if necessary. The experimental arrangement for the flow system is shown in (B). A 1.5 l fermentor (Tokyo Rikakikai) which equipped with electrodes of $O_2$, $CO_2$ and pH was used.

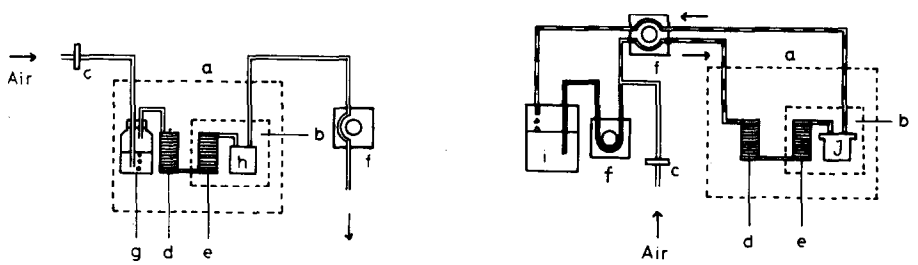


Fig. 3. Culture systems.
(a),air bath; (b),calorimeter; (c),air filter; (d),outer heat exchanger; (e),inner heat exchanger; (f),peristaltic pump; (g),humidifier; (h),batch vessel; (i),fermentor; (j),flow vessel.

Cultures were pumped by a peristaltic pump to a T-piece where it was met by a constant flow of air. The mixed flow of culture and air was taken through the calorimeter and from there return to the fermentor. During the experiment the concentration of $O_2$ and $CO_2$ were measured and pH was kept constant at 5.0 in the fermentor.

Strain and culture medium

The yeast used in this study is a strain of Saccharomyces cerevisiae kindly provided by Dr. Gunge with which studies has been reported previously (refs.1, 4,5) and other test fungi are Aspergillus oryzae IAM 2630, Aspergillus niger IAM 2093 and Aspergillus niger IAM 2094. Unless otherwise stated Kilkenny and Hinshelwood's synthetic medium (ref.6) of which glucose concentration was adjusted to 0.2% was used as a growth medium.

Chemical analysis

Glucose was determined enzymatically using the Glucostat method and ethanol was determined by gas chromatography. $O_2$ concentration and $CO_2$ concentration were measured by using electrodes (Oriental Electric Co.). Turbidity of the culture was measured at 550 nm with a Shimadzu Spectronic 20 spectrophotometer.

## RESULTS AND DISCUSSION

Electrical calibration

The electrical calibration data were obtained by electrically heating at 5 mW for different periods with concomitant rotation of the calorimeter at 50 r.p.m.. For the calibration of the batch system, batch vessels were filled with 4.5 ml of distilled water and the sample vessel was aerated with the rate of 300 ml/hour. The relationship between the heat input and the chart area of calorimetric records was proportional and the heat effect sensitivity was 0.12 mV/mW and the time constant was 4.5 min. Calibration for the flow system was carried out by using the flow vessel throguh which distilled water and air were flowing and the reference vessel was charged with 1.4 ml of water. The flow rate of water and air were 20 ml/hour and 30 ml/hour respectively. The sample vessel was heated at 1 mW for different periods. Results showed that the heat input and the chart area was proportional and the heat effect sensitivity was 0.11 mV/mW and the time constant was 2.1 min.

The effects of the flow rate of air and water on the heat effect were tested by electrical calibration. The difference in the aeration shows no effect whereas the increase of flow rate of water shows the decrease of the heat effect, however, the effect is small and no significant error will arise if the flow rate is kept constant during the experiment.

Yeast growth with the batch system

For the experiment with the batch system, the active vessel was charged with 4.0 ml of the medium and 0.5 ml of the inoculum separately. The vessel was placed in the vessel holder and aeration was started immediately after the connection with the aeration line. After the temperature equilibration, the calorimetric measurment was started by rotating calorimeter, thus the medium and the inoculum were mixed. The power-time (p-t) curve obtained from the experiment is shown in Fig. 4. The pattern of p-t curve indicates that yeast cells grew under sufficient aerobic conditions in the vessel by comparison with the previous result (ref.1).

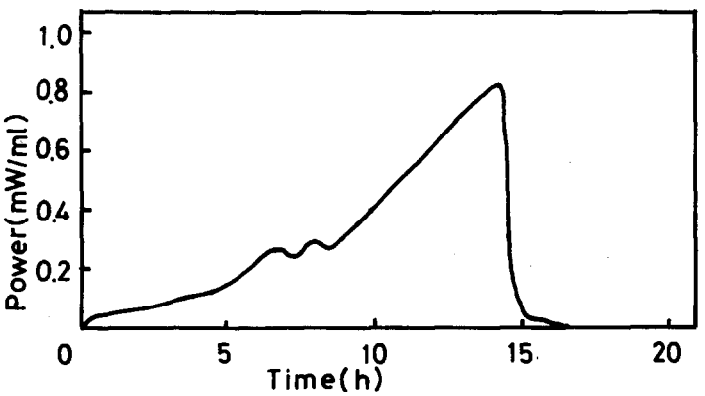


Fig. 4. Power-time curve of aerobic growth of S. cerevisiae by the batch system.

Yeast growth with the flow system

Chemical analyses of the culture were carried out during the flow calorimetric experiment (Fig. 5). Before begining of the experiment, the fermentor was filled with 1.2 l of the medium, then the medium was circulated through the arrangement concomitant rotation of the calorimeter. The experiment was started by adding 10 ml of the inoculum to the fermentor after the thermal equlibration and the culture in the fermentor was aerated at the rate of 500 ml/min and agitated by the stirrer at 30 r.p.m.. During experiments the samples were taken out from the fermentor every one hour for analyses of glucose, ethanol and cell concentrations. $O_2$ concentrations were measured in the fermentor and the effluent from the calorimeter and $CO_2$ concentration in the fermentor was measured during the calorimetric experiments. As shown in Fig. 5 the curves of $O_2$ concentrations show that the culture was aerated sufficiently through the experiments. The p-t curve had two peaks which indicate the diauxie and the first

peak was identified as the growth on glucose and the second peak was due to the consumption of ethanol. We may also note that the peaks of the $CO_2$ curve correspond with those of the p-t curve.

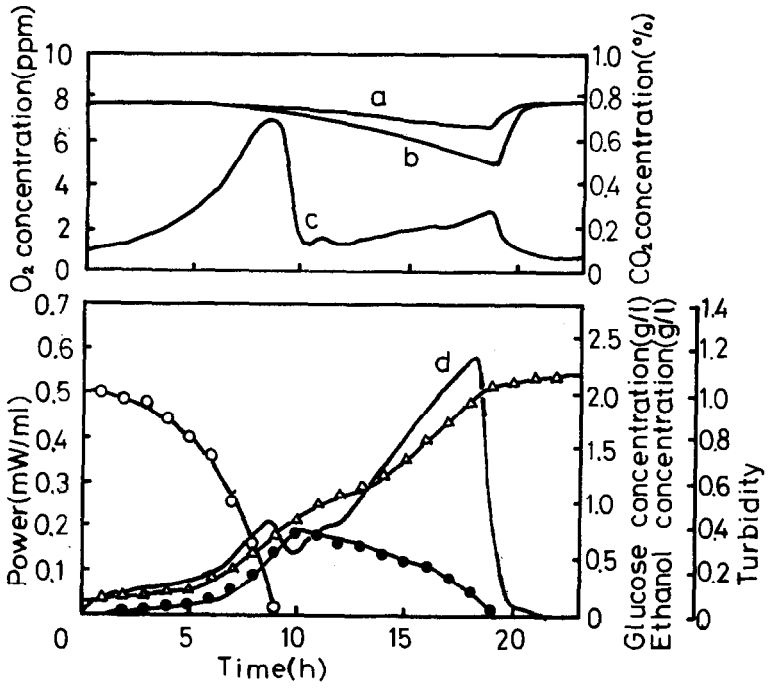


Fig. 5. Aerobic growth experiments with S. cerevisiae by the flow system. (a),$O_2$ concentration of the effluent; (b),$O_2$ concentration in the fermentor; (c),$CO_2$ concentration in the fermentor; (d),power-time curve; ○,glucose concentration; ●,ethanol concentration; △,turbidity.

Continuous culture of yeast

Calorimetry of continuous culture has an advantage that a microbial culture is kept under the constant condition for long time by controlling the dilution rate (D) and glucose concentration in the medium. Calorimetric sutdies of the chemostat continuous culture of yeast were carried out under four different conditions. As shown in Fig. 6, stable p-t curves were obtained with each of the culture conditions. Heat production per formed biomass were varied with the culture conditions. The results also show that there were large difference in the various metabolic parameters, however, further studies are necessary to solved this problem.

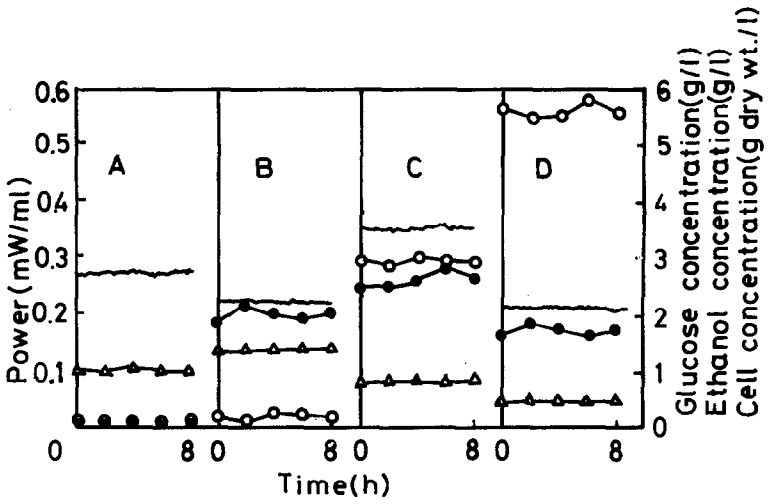


Fig. 6. Aerobic growth experiments with continuous culture of S. cerevisiae under different conditions.
(A) glucose,0.2%; D,0.05$h^{-1}$. (B) glucose,1.0%; D,0.10$h^{-1}$.
(C) glucose,1.0%; D,0.15$h^{-1}$. (D) glucose,1.0%; D,0.20$h^{-1}$.
———,power-time curve; ●,ethanol concentration; ○,glucose concentration; ▲,cell concentration.

Growth experiment with immobilized yeast cells

Recently several investigations have been carried out on the utility of the immobilized yeast for ethanol fermentation (ref.7), however, no effective method for measuring the activity of immobilized yeast cells has been established. Calorimetric studies seem to be available for this purpose. The test flow calorimetry were carried out by using spherical agar gel beads (a diameter of 4 mm) which contain $10^5$ yeast cells/ml of gel. Gel beads were prepared by the method of Sato et. al (ref.8). Five ml of the beads were packed in the flow vessel and the medium was supplied to the vessel with air. Glucose, ethanol and cell concentrations of the effluent from the calorimeter were measured during the experiment. As shown in Fig. 7, until about 30 hour the rate of heat production increased exponentially and the steady state continued from 60 hour to 120 hour. The heat production per formed biomass was 8.1 (J/mg dry wt.) which was much the same as that with yeast grown in continuous culture. We may also note that the p-t cruves agree well qualitatively with the curves of the other metabolic parameter.

Growth experiment with fungi

Calorimetry with fungi was carried out by the batch system. The batch vessel

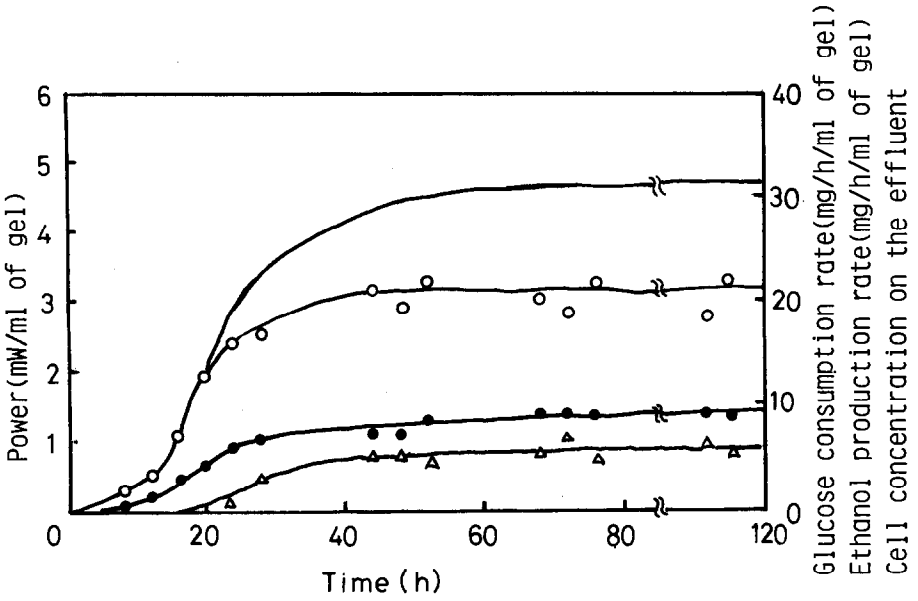


Fig. 7. Time course of growth experiments with immobilized S. cerevisiae by continuous flow system.
———, power-time curve; o, glucose consumption rate; ●, ethanol production rate; △, cell concentration of the effluent.

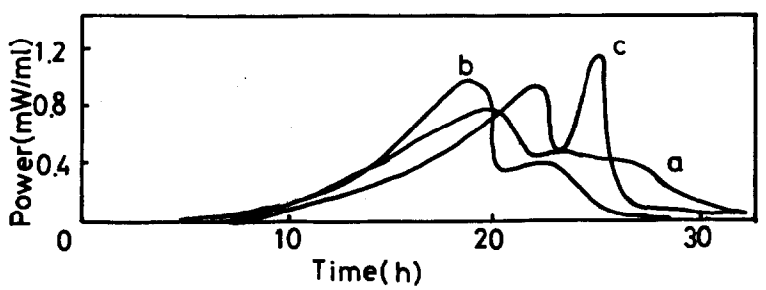


Fig. 8. Power-time curves of growth experiments with different fungi.
(a), A. niger IAM 2093; (b), A. niger IAM 2094; (c), A. oryzae IAM 2630.

was filled with 2.0 ml of the medium and 0.5 ml of a conidia suspension as a inoculum. The conidia suspension was obtained by washing a slant culture with distilled water. Fig. 8 shows the p-t curves with the fungi growth. Each of the p-t curves showed two peaks and the heat production per glucose consumed were 1240 (KJ/mol) for A. niger IAM 2093, 1220 (KJ/mol) for A. niger IAM 2094 and

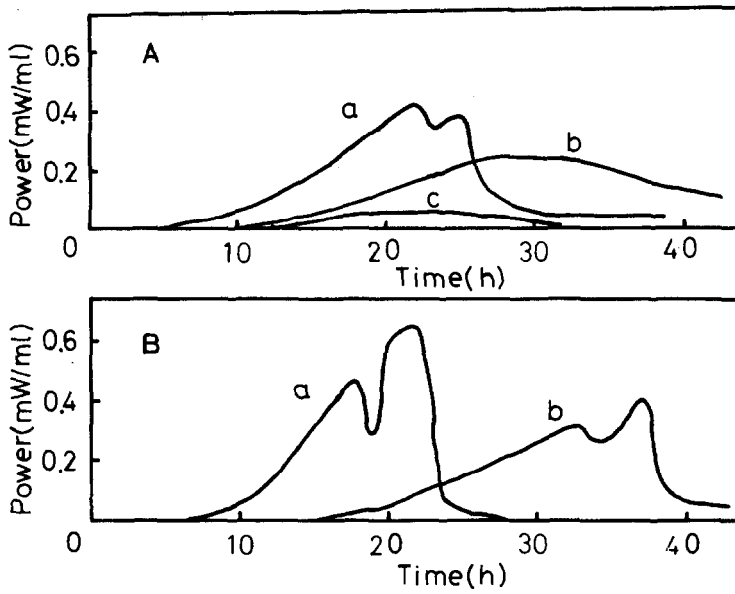


Fig. 9. Effects of TCA cycle inhibitors on the growth of A. oryzae.
(A) Effect of fluoroacetate. (a),0.1 mM ; (b),1 mM; (c),10 mM.
(B) Effect of malonate. (a),1 mM; (b),10 mM.

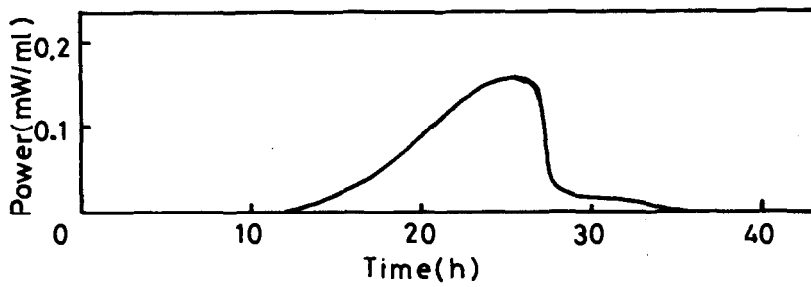


Fig. 10. Power-time courve of growth experiment with A. oryzae in a citrate medium.

1260 (KJ/mol) for A. oryzae IAM 2630 respectively. To elucidate the nature of the first peak of the p-t curve, the following experiments were carried out in which metabolism was inhibited by TCA cycle inhibitors (Fig. 9) or glucose was replaced by citrate (Fig. 10), because these fungi known to accumulate citrate during the growth (ref.9). The first peak of the p-t curves disappeard when 1 mM of fluoroacetate (the inhibitor of aconitase) was added (A) whereas

the first peak still observed with malonate (the inhibitor of succinate dehydrogenase), (B). Fig. 10 shows the p-t curve with a single peak for the fungi growth in the citrate medium. From these results, it seems probable that the first peak of the p-t curve was correlated with the conversion of glucose to citrate and the second peak seemd to be due to the degradation of citrate in the medium.

## CONCLUSION

The calorimeter described in this paper have proved to be useful for large microbial cellular systems. To extent the use of this calorimeter systems for the other cellular systems, it is necessary to make improvements on the instrumental properties and working processes.

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