Design of a Calorimeter for the Continuous Study of Heat Production during Anaerobic Microbial Growth

Katsutada TAKAHASHI

Laboratory of Biophysical Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka

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A conduction type calorimeter has been designed to chase microbial growth in batch system. The calorimeter is of a twin structure having thermopile plates as a temperature sensor. The heat evolution during the microbial growth at a required temperature can be observed as an output-voltage generated at thermopile terminals with a sensitivity of 58.5 mV K\(^{-1}\).

A stainless steel cell with a volume of 300 cm\(^3\) serves as a culture cell which is capable of being autoclaved prior to the initiation of calorimetric run, taking out from the calorimeter body.

Because of the twin structure, the apparatus works with sufficient stability in detecting small heat evolution for long duration. Its operation has been demonstrated with the growth of Sacch. cerevisiae grown on liquid synthetic medium under anaerobic condition.

Recent development in calorimetric techniques has enabled the analytical application of calorimetry to the various microbial systems. Most of the works in this area have been made with the Tian-Calvet type calorimeter or the microcalorimeter designed by Wadsö, both being marketed commercially.

However, the capacity of calorimeter cell of these existing designs is so small (5 to 30 cm\(^3\)) that their use for the microbiology is often inconvenient as to the quantitative analysis of biochemical processes occurring in the system. Therefore, it seems desirable to construct a new calorimeter with a cell capacity of 50 to 300 cm\(^3\) which is suitable for the purpose of chasing more quantitatively the thermal process of microbial system. In the present paper, design and testing of a high sensitive calorimeter especially designed for the above aim are described.

**Calorimeter and culture cell**

The calorimeter is in principle a version of the heat conduction type microcalorimeter designed by Amaya and Hagiwara, where the calorimeter reaction cell is replaced by a culture cell equipped with a stirrer and an inoculation tube.

The calorimetric operation is such that: Heat evolved during the microbial growth in the culture cell is conducted to a surrounding heatsink (thermostated metal block) through a thermopile which is situated between the cell and the heatsink. The heat flow thus taking place between the two is measured as a thermopile voltage-time curve. The voltage derived is directly proportional to a heat evolution rate, when the temperature of heatsink is constant and the heat conduction is in the steady state.

In the calorimeter two identical cells are placed to give a twin structure and the differential voltage from the two is taken as a calorimeter output-signal.

The apparatus is schematically shown in Fig. 1. In an aluminum heatsink with dimensions of 310 × 210 × 200 cm (A) two cylindrical copper cans (B) with a diameter of 7.2 cm, a depth of 10 cm and a wall thickness of 0.4 cm are arranged at a symmetrical position. These two cans serve as a container of calorimeter cell; one for culture cell and the other for reference cell.

Between the can and the heatsink 9 semiconducting thermopile plates (C) are placed in good thermal contact with the wall of both the
FIG. 1. Structure of Calorimeter.

can and the heatsink; one at the bottom and the other eight on the lateral face of the can as seen in Fig. 1. Thermopile plate used is Thermo-electric module HTM-0516 (Sharp Co., Osaka) which contains 16 pairs of P and N semiconducting elements and generates 6.5 mV terminal voltage when a temperature difference of 1 K exists between both sides of the plate. Terminals of the 9 thermopile plates are connected in series to form a thermopile unit with a sensitivity of 58.5 mV K\(^{-1}\) for each can and the two thermopile units thus formed are connected in opposition so that the differential voltage from the two is lead out.

To the inner wall of the can a calibration heater (D) made of 200.2 ohm manganine wire is attached and is used for the electrical calibration of the system.

Temperature of the heatsink is regulated by circulating water through a copper spiral tube (E) where the temperature of water is thermostated within ±0.02 K by Thermo-electric circulating bath TE-12 K (Sharp Co., Osaka). Thus the calorimetric run can be performed at a required temperature.

The entire calorimeter body is thermally insulated on the outside by a styrofoam adiabatic-shield which forms an insulation box with outside dimensions of 800 × 850 × 700 cm.

A culture cell (F) made of stainless steel has a volume of 300 cm\(^3\). A cylindrical vessel with a diameter of 8 cm and a depth of 14 cm provided with a screw lid having a stirrer (G) and an inoculation tube (H) serves as the cell*. As for the study of aerobic growth, a specified culture cell equipped with aeration system may be used. However, the calorimetric operation of aerobic growth is technically more difficult than that of anaerobic growth, since aeration is accompanied by a large endothermic heat due to vaporization of water which affects on the thermal data. Design and testing of aerobic version of the present apparatus will be reported in later paper.
One more identical cell is prepared which is used as the reference cell.

The stirrer is of a propeller type with a diameter of 4 cm and the upper end of its shaft is connected via a flexible joint to a synchronous motor with 200 rpm (Model SM8P10, Japan Servo Co., Tokyo) which is supported on the top plate of calorimeter insulation box.

After the cells containing culture medium are autoclaved, they are placed in the cans containing 40 ml of liquid paraffine. In a space between the cell and the can a layer of liquid paraffine (I) is formed which acts as a heat conductor between the two. When the thermal equilibrium is attained as judged from the chart record of calorimeter output-voltage, the inoculation is performed on the culture cell through the inoculation tube by a sterilized syringe and the calorimeter signal is recorded as a voltage-time curve.

The calorimeter body, the culture cell and the over-all view of assembly are shown in Figs. 2, 3 and 4, respectively.

When the whole assembly is placed in a
thermostated room maintained at 24±0.3°C, the stability of calorimeter expressed as an apparent temperature difference between the two cells is within 0.00001 K.

**Calibration of the calorimeter**

The calibration test was performed by sending known electric current to the calibration heater. Measurements were made on the calorimeter signal at various electrical heating rates over the same range as occurred in the thermal process during the microbial growth being studied. The calorimeter output-voltage at a steady state of constant heating was plotted against the heating rate. As shown in Fig. 5 a linear relationship between the two was observed. Thus the present construction meets the requirement of a proportionality in the calorimeter signal to the heat evolution rate. From the above relationship the voltage coefficient of calorimeter was obtained to be 0.01855 mV J⁻¹. Heat leakage modulus...
(cooling constant) of the system determined from the Newton's law\(^{13}\) was 1.69 H\(^{-1}\).

**Performance with yeast growth**

Heat evolution during the yeast growth was studied as a demonstration. Figure 6 shows a typical example of thermograms (chart record of calorimeter signal) observed for the anaerobic growth of *Sacch. cerevisiae* grown on a liquid synthetic medium containing 0.3% glucose. On the surface of the medium in the cell a liquid paraffine layer with a thickness of 3 cm was placed in order to prevent the dissolution of air-oxygen. Change in turbidity of the medium measured separately under the same condition with that employed for the calorimetric run is also given for comparison. From Fig. 6 it seems likely that the thermogram observed gives the derived function of the growth curve as judged from the turbidity measurement. This agrees with the observations reported in literature.\(^{3-5}\) Quantitative analyses of the growth thermogram will be made in later paper.\(^{14}\)

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**REFERENCES**


