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**Edited by A. Fiechter**

**J. H. Luong, B. Volesky**

**Heat Evolution During the Microbial Process-  
Estimation, Measurement, and Applications**

**F. Parisi**

**Energy Balances for Ethanol as a Fuel**

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**Biotechnology of Thermophilic Bacteria-Growth,  
Products and Application**

**E. N. Kondratieva, I. N. Gogotov**

**Production of Molecular Hydrogen in  
Microorganisms**

# Microbial Activities

With Contributions by  
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J. H. Luong, F. Parisi,  
B. Sonnleitner, B. Volesky

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# Heat Evolution During the Microbial Process — Estimation, Measurement, and Applications

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Estimation and measurement techniques of microbial heat evolution during a microbial process are concisely discussed in this review.

In a well-defined medium and when the products are specified, the heat evolution quantity can be estimated directly from the heats of combustion of organic substrate, products, and biomass. Theoretical estimations of the heats of combustion of organic substrates or products can be very accurate. The heat of combustion of microbial cells is usually determined by burning microbial cells in an oxygen bomb calorimeter. However, when the composition of the biomass is known, the heat of combustion of the cell can be calculated by one of the following procedures: the Giese method, the Dulong equation, and the method of Mennett and Nakayama.

In a complex medium and when either non-cellular or cellular products are not completely specified, heat evolved during the microbial activity can be directly measured by microcalorimetry, dynamic calorimetry, and continuous calorimetry, respectively. Microcalorimetry usually involves the use of an adiabatic calorimeter, a thermal fluxmeter or a flow calorimeter.

On a bench-scale, dynamic calorimetry of continuous calorimetry can be applied to monitor the quantity of heat evolution during the fermentation. While the continuous calorimetric technique is used to continuously measure the heat evolution, dynamic calorimetry only provides intermittent data and requires continuous attention during the course of the process.

Based upon the heat evolution parameter; the biomass concentration, the oxygen consumption, the carbon dioxide production, and the substrate utilization, the energy requirements for growth, product formation and maintenance purposes are correlated to the biomass energetic yield and the product energetic yield. Under aerobic conditions, the fraction of the substrate energy evolved as heat is equal to the fraction of available electrons transferred to oxygen. As a consequence, either the heat evolution data or the oxygen consumption data is quite useful for monitoring a microbial process.

The heat evolution data can be used as a kind of a "heat probe" employed in process control and in on-line optimization of either aerobic or anaerobic systems. The heat evolution should be used together with the other microbial process parameters for estimating the cellular metabolic activity and bioreaction kinetics and for checking the consistency of other experimental data collected during the experiment.

## 1 Introduction

The growth of microorganisms is accompanied by the production of heat, regardless of whether the system is aerobic or anaerobic or whether the final product is biomass or metabolites. A large part of the heat is generated during the degradation of the organic substrate, which serves as a carbon and energy source. The catabolic process is always associated with a significant free-energy decrease. Part of the released energy is conserved in high-energy bonds of adenosine triphosphate (ATP) or other energy-storage compounds, providing energy for biosynthesis of cellular components and other microbial activities as required. The rest of the substrate's original energy is released as heat. In addition to the heat production during the catabolism of the energy-rich nutrient molecules, most of the ATP energy is also liberated as heat during its utilization in the cellular activities, providing support for the microbial growth and other cell functions.

The amount of heat produced is dependent upon the type of catabolic pathway through which the organic substrate is metabolized. It is also dependent upon the energetic coupling of energy-storage compound (ATP, etc.) generation and cell biosynthesis. Variations in the microbial heat parameter reflect the type of metabolic activities of the cell, as well as the degree of perfection with which the cellular metabolism and anabolism are completed. This indicates that the heat generation during the activity of microbial culture may be used to evaluate the regulatory mechanism of the cellular energy metabolism. It may also be very helpful in estimating the energetic efficiency of catabolic pathways, the heat energetic yield and the efficiency of energy recovery by the cells.

However, the metabolic heat is still an under-utilized parameter for assessing the degree of microbial activities. This could be explained by the fact that the heat evolution is not usually monitored due to the difficulties and/or complexities involved in the measurement of such an elusive parameter. Also, there is a lack of good interpretation of the heat evolution quantity. In most cases, the rate of metabolic heat release has been correlated with the rate of oxygen consumption by the growing culture<sup>1,2,3,4</sup>). This type of correlation is not applicable to anaerobic microbial systems and it only represents a narrow aspect of cellular metabolic activity.



A successful attempt was reported by Belaich et al.<sup>5)</sup> to correlate the quantity of heat released with the quantities of substrate utilization and product (ethanol) biosynthesis. The metabolic heat parameter has also been used to predict the maximum value of the specific rate of substrate utilization and the Michaelis-Menten constant of the ethanol biosynthesis system.

Many investigators have attempted the correlation between the heat evolution and cellular metabolic activity as a means of gaining insight into the thermodynamics and energetics of growth<sup>6,7)</sup>. However, little attention has been given to the possibility of measuring heat production for routine assessment of microbial kinetics. Mou and Cooney<sup>8)</sup>, applying the dynamic calorimetric technique to an antibiotic biosynthesis in complex media, found thermal measurements useful for monitoring cell growth and as a physiological parameter throughout the bioconversion process.

Recently, Wang et al.<sup>9)</sup>, Luong and Volesky<sup>10)</sup>, Volesky et al.<sup>11)</sup> have used the parameter of heat produced to indirectly assess the concentration and rate of growth of cells during a microbial process.

Heat and mass balances have been presented together with three regularities which Minkevich and Eroshin<sup>12,13,14,15)</sup> have identified and quantified. Erickson<sup>16,17)</sup>, and Erickson and co-workers<sup>18,19,20,21)</sup> have applied the heat and mass balance regularities to specific culture conditions such as continuous cultivations, batch biosystems, and processes with extracellular products. The above-mentioned investigators have demonstrated that material and energy balance regularities can be used to analyze the process energetics.

Applications of material and energy balances and associated regularities in on-line data analysis have been attempted<sup>22)</sup>. This approach is particularly important in on-line data analysis where only limited microbial process data are available.

In view of this, the main purpose of this paper is to illustrate some important applications of the heat evolution quantity as a microbial process parameter. In aerobic systems, it can be used to predict the cellular metabolic activity, the biomass concentration, the oxygen uptake rate, and the organic substrate utilization rate. The heat evolution parameter can also be used, together with either oxygen uptake data or carbon dioxide respiration data, for checking the consistency of experimental data collected during the experiment.

In anaerobic systems, the applicability of the heat evolution data as an analytical tool for the routine assessment of microbial kinetics will be discussed. Theoretical estimation and experimental measurements of the heat evolution data will also be presented in detail.

## 2 Theoretical Calculation of Microbial Metabolic Heat Evolution

The microbial heat evolution,  $\Delta Q$ , from an exothermic microbial process is calculated as follows:

$$\Delta Q = (-\Delta H_s)(-\Delta S) + (-\Delta H_n)(-\Delta N) - (-\Delta H_c)(\Delta X) - \sum(-\Delta H_{pi})(\Delta P_i) \quad (1)$$

where

- $-\Delta H_s$  = heat of combustion of carbon substrate
- $-\Delta H_n$  = heat of combustion of nitrogen source
- $-\Delta H_c$  = heat of combustion of microbial cells
- $-\Delta H_{pi}$  = heat of combustion of  $i^{\text{th}}$  product
- $-\Delta S$  = amount of substrate utilized
- $-\Delta N$  = amount of nitrogen utilized
- $\Delta X$  = amount of microbial cells produced
- $\Delta P_i$  = amount of  $i^{\text{th}}$  product produced

The combustion heat values of various substances,  $(-\Delta H_s)$  and  $(-\Delta H_{pi})$  can be obtained from many different sources. In the absence of experimental data, the heat of combustion of an organic substance can be calculated by the following procedures<sup>23)</sup>:

— The electrons in the C—C bond or C—H bond of a respective molecule produce heat energy of 108.99 kJ per equivalent of electrons.

— The electrons in C = O, CHOH, and CH<sub>2</sub> OH in the respective molecule produce additional heat energy of 81.59, 54.39, and 54.39 kJ per equivalent of electrons respectively.

Generally, the values predicted on the basis of the above assumption appear to compare well with the experimental values (Table 1). The heat of combustion of microbial cells  $(-\Delta H_c)$  is usually unknown. It is experimentally determined by burning

**Table 1.** The values of heat of combustion of some chemicals theoretical vs experimental

Chemical	Heat of combustion (kJ mol <sup>-1</sup> )		% Difference from the experimental value
	Measurement <sup>24)</sup>	Predicted	
Methane	890.3		— 2.07
Methanol	726.8	708.3 (108.99 × 6 + 54.39)	— 2.53
Ethanol	1366.1	1362.3 (108.99 × 12 + 54.39)	— 2.76 × 10 <sup>-1</sup>
Glycerol	1664.4	1689.1 (108.99 × 14 + 54.9 × 3)	1.48
Formaldehyde	561.1	517.6 (108.99 × 4 + 81.59)	— 7.75
Acetaldehyde	1166.5	1171.5 (108.99 × 10 + 81.59)	4.3 × 10 <sup>-1</sup>
Acetone	1825.5	1829.7 (108.99 × 16 + 81.59)	2.29 × 10 <sup>-1</sup>
Formic acid	263.2	218 (108.99 × 2)	— 17.17
Acetic acid	872.8	871.9 (108.99 × 8)	— 9.6 × 10 <sup>-2</sup>
Lactic acid	1364	1362.3 (108.99 × 12 + 54.39)	— 1.13 × 10 <sup>-1</sup>
Pyruvic acid	1171.5	1171.5 (108.99 × 10 + 81.59)	0
Tartaric acid	1151	1198.7 (108.99 × 10 + 54.39 × 2)	4.14
Maleic acid	1338.9	1362.3 (108.99 × 12 + 54.39)	1.72
Succinic acid	1494.1	1525.9 (108.99 × 14)	2.13
Fumaric acid	1338.9	1305.4 (108.99 × 12)	— 2.5
Xylose	2349.3	2355.6 (108.99 × 20)	0.27
Galactose	2806.2	2887.8 (108.99 × 24 + 54.39 × 5)	2.91
Glucose	2815.8	2887.8 (108.99 × 24 + 54.39 × 5)	2.56
Rhamnose	3005.4	3050.1 (108.99 × 26 + 54.39 × 4)	1.49
Maltose	5649.2	5666.8 (108.99 × 48 + 54.39 × 8)	3.11 × 10 <sup>-1</sup>

microbial cells in an oxygen bomb calorimeter. According to Prochazka et al.<sup>25)</sup>, the mean calorific content of the various microorganisms they tested was around 22.6 kJ per g cells on an ash-free, dry weight basis; the range of variation was from 20.9 to 25.1 kJ per g cells.

When the composition of the cells is specified, the heat of combustion of the cells can be calculated by the following methods:

1. A revised version of the Dulong equation<sup>26)</sup> can be used to calculate the calorific content of microorganisms.

$$\frac{\text{kJ}}{\text{g cells}} = 33.76C + 144.05 \left[ H - \frac{O}{8} \right] \quad (2)$$

Where C, H, and O are the weight fractions of carbon, hydrogen and oxygen of the microbial cells.

2. A similar equation used by Giese<sup>27)</sup> to calculate the heat of combustion of an organic compound can be applied to calculate the calorific content of microorganisms.

$$\text{kJ g}^{-1} = \frac{460.24 \times (\text{RL}) \times N_c}{\text{mol. wt. of cells}} \quad (3)$$

Where the reduction level of the cells (RL) is expressed by the following formula:

$$\text{RL} = \frac{2N_c + (N_H/2) - N_O}{2N_c} \quad (4)$$

$N_c$ ,  $N_H$ , and  $N_O$  are the numbers of carbon, hydrogen, and oxygen atoms in an empirical molecular formula for cells.

The Giese equation appears to be more applicable than the Dulong equation for estimating the calorific content of microbial cells<sup>26)</sup>.

3. An interesting method uses also developed by Mennett and Nakayama<sup>28)</sup> to estimate the calorific content of microorganisms. This method is based upon the sum of the heats of combustion of the macromolecular components of the cells: 22.59 kJ per g protein 38.9 kJ per g lipid, 17.57 kJ per g carbohydrate and 14.64 kJ per g nucleic acids. This theoretical analysis estimated a value of 22.17 kJ per g cells of ash-free cells, dry weight for *Pseudomonas fluorescens* which agreed very well with the experimental value obtained by Prochazka et al.<sup>25)</sup>.

### 3 Estimation of the Microbial Metabolic Heat Evolution Based on Respiration

In the cases of aerobic cultivation without producing noncellular product, i.e.  $\Sigma \Delta P_i = 0$ , the following equation can be rewritten based upon Eq. (1):

$$\Delta Q = (-\Delta H_s)(-\Delta S) + (-\Delta H_n)(-\Delta N) - (-\Delta H_c)(\Delta X) \quad (5)$$

Minkevich and Eroshin <sup>2)</sup> found that the heat of combustion of an organic substance and dried could be calculated by multiplying the proportionality constant of 451.8 kJ per mol O<sub>2</sub> by the amount of oxygen required for the oxidation of each substance.

$$(-\Delta H_s) = (-\Delta H_0) A_s \quad (6)$$

$$(-\Delta H_c) = (-\Delta H_0) A_c \quad (7)$$

where

$$(-\Delta H_0) = 451.8 \text{ kJ per mol O}_2 \quad (8)$$

If it is further assumed that the heat of combustion of nitrogenous substance could also be estimated similarly:

$$(-\Delta H_n) = A_n(-\Delta H_0) \quad (9)$$

from Equations (5)–(9), it follows that:

$$\Delta Q = [A_s(-\Delta S) + A_n(-\Delta N) - A_c(\Delta X)](-\Delta H_0) \quad (10)$$

obviously,

$$(-\Delta O_2) = A_s(-\Delta S) + A_n(-\Delta N) - A_c(\Delta X) \quad (11)$$

where  $(-\Delta O_2)$  = Quantity of oxygen required for aerobic respiration

Therefore,

$$\Delta Q = (-\Delta H_0) (-\Delta O_2) \quad (12)$$

Cooney et al. <sup>1)</sup>, using the *in situ* dynamic calorimetric technique directly measuring the metabolic heat and the oxygen uptake rate, found the following relationship:

$$\Delta Q = (518.8 \text{ kJ per mol O}_2) (-\Delta O_2) \quad (13)$$

Luong and Volesky <sup>4)</sup>, developing the continuous calorimetric technique of directly measuring the heat evolution of *A. niger*, *E. coli*, *C. lipolytica*, *C. intermedia*, and *C. utilis*, found the following correlation:

$$\Delta Q = (460.2 \text{ kJ per mol O}_2) (-\Delta O_2) \quad (14)$$

Based on the concept of Mayberry et al. <sup>29)</sup> and Payne <sup>30)</sup> who advocated the use of heat of combustion per available electron equivalent, Imanaka and Aiba <sup>3)</sup> developed the following relationship:

$$\Delta Q = (-\Delta H_0^*) (-\Delta O_2) \quad (15)$$

where the value of  $(-\Delta H_0^*)$  may be taken as 111 (kJ) per av.e)  $\times 4$  (av.e per mol  $O_2$ ) = 444 kJ per mol  $O_2$ .

The rate of oxygen consumption is calculated from the mass balances of respective gas between inlet and outlet air streams of a bioreactor <sup>31)</sup>:

$$\dot{q}_{O_2} = \frac{F_N}{V_L} \left[ \frac{P_{O_2}^{in}}{P_T - P_{O_2}^{in} - P_{CO_2}^{in} - P_w^{in}} - \frac{P_{O_2}^{out}}{P_T - P_{O_2}^{out} - P_{CO_2}^{out} - P_w^{out}} \right] \quad (16)$$

where

- $P_T$  = total pressure
- $P_{O_2}^{in}, P_{O_2}^{out}$  = partial pressure of oxygen in inlet and outlet gas
- $P_{CO_2}^{in}, P_{CO_2}^{out}$  = partial pressure of carbon dioxide in inlet and outlet gas
- $P_w^{in}, P_w^{out}$  = partial pressure of water in inlet and outlet gas
- $F_N$  = molal flow rate of inert gas ( $N_2$ )
- $V_L$  = volume of liquid broth

The total oxygen consumed during a microbial process is then calculated by an integrating method:

$$(-\Delta O_2) = \int_0^t \dot{q}_{O_2} dt \quad (17)$$

In the absence of experimental data and when the elemental compositions of microbial cells are specified, the amount of oxygen required per unit weight of microbial cells produced can be estimated by the following formula <sup>32)</sup>:

$$\frac{g O_2}{g \text{ cell}} = 16 \left[ \frac{2C + (H/2) - O}{Y_{x/s} (\text{Mol. wt.})} + \frac{O'}{1600} - \frac{C'}{600} + \frac{N'}{933} - \frac{H'}{200} \right] \quad (18)$$

where

- C, H, and O represent the number of atoms of carbon, hydrogen, and oxygen, respectively, in each molecule of carbon source.
- C', H', O', and N' represent the percentage of carbon, hydrogen, oxygen, and nitrogen, respectively in the cells.
- Mol. wt. represents the molecular weight of the carbon source.
- $Y_{x/s} = \Delta X / -\Delta S$ , represents the yield of cells based on carbon source.

Equation (18) is only valid when the nitrogen source is ammonia and the only products of metabolisms are the cells, carbon dioxide, and water. Growth yield based upon oxygen,  $Y_{x/O} = \Delta X / (-\Delta O_2)$ , gram cell produced per gram of oxygen consumed of various microorganisms growing aerobically in minimal media is summarized in Table 2.

#### 4 Direct Measurement of the Microbial Metabolic Heat Evolution

A literature review indicated that much of the earlier work concerning the measurement of metabolic heat involved the use of crude calorimeters. Other techniques have also been used to measure the microbial heat release <sup>42,43,44)</sup>. In most cases, the

**Table 2.** Values of  $Y_{x/o}$  of various microorganisms growing aerobically in minimal media (presumably without producing noncellular products)

Microorganism	Substrate	$Y_{x/o}$ (g g <sup>-1</sup> )
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Maltose	1.50
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Mannitol	1.18
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Fructose	0.42
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Glucose	0.40
<i>Candida utilis</i> <sup>34)</sup>	Glucose	1.32
<i>Penicillium chrysogenum</i> <sup>35)</sup>	Glucose	1.35
<i>Pseudomonas fluorescens</i> <sup>34)</sup>	Glucose	0.85
<i>Rhodopseudomonas spheroides</i> <sup>34)</sup>	Glucose	1.46
<i>Saccharomyces cerevisiae</i> <sup>36)</sup>	Glucose	0.97
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Ribose	0.98
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Succinate	0.62
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Glycerol	0.97
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Lactate	0.37
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Pyruvate	0.48
<i>Aerobacter aerogenes</i> <sup>33)</sup>	Acetate	0.31
<i>Candida utilis</i> <sup>34)</sup>	Acetate	0.70
<i>Pseudomonas fluorescens</i> <sup>34)</sup>	Acetate	0.46
<i>Candida utilis</i> <sup>34)</sup>	Ethanol	0.61
<i>Pseudomonas fluorescens</i> <sup>34)</sup>	Ethanol	0.42
<i>Klebsiella</i> sp. <sup>38)</sup>	Methanol	0.56
<i>Methylomonas</i> sp. <sup>39)</sup>	Methanol	0.53
<i>Pseudomonas</i> sp. <sup>40)</sup>	Methane	0.20
<i>Pseudomonas</i> sp. <sup>41)</sup>	Methane	0.19
<i>Pseudomonas methanica</i> <sup>41)</sup>	Methane	0.17

techniques require relatively complicated apparatus and/or procedures for determining the heat produced during growth and product formation.

#### 4.1 Microcalorimetry

The literature contains a very large number of descriptions of calorimeters. General principles of design and operation of reaction calorimeters have been discussed by Skinner et al.<sup>45)</sup> In this review, discussion is restricted to a few representative instruments satisfactory for microbial studies. It is worthy of mention that microcalorimetry is the study of small heat changes, not necessarily with small quantities of material.

The biggest task in the investigation of microbial reaction by calorimeters is to maintain instrumental stability for long periods. In order to achieve this objective, calorimeters should operate on the twin calorimeter system introduced by Joule. Measurements are made by comparison of the temperature or some function of it between two calorimeters. As a result, long-term drifts can be neglected since both bioreactor vessels are equally affected.

##### 4.1.1 Adiabatic Calorimeters

Adiabatic calorimeters consist of one reaction vessel and one balance vessel. These vessels are made as nearly identical as possible and contained within the same jacket.

The vessels are well insulated from their environment to minimize the experimental error due to heat leakage and the maximum possible temperature rise is produced in the reaction vessel. The temperature sensors are usually thermistor or resistance thermometers.

During the experiment, electrical heating is applied to the matching balance vessel so that it maintains the same temperature as the reaction vessel. Heating is also arranged to operate continuously through a feedback control circuit so that any heat produced by microbial metabolism in the reaction vessel is continuously balanced by electrical heating of the reference balance vessel. The quantity of electrical heating is continuously recorded but temperature is not measured directly.

In adiabatic calorimeters, the electrical power supplied is calculated as follows:

$$Q = \int_0^t \frac{V^2}{R} dt \quad (19)$$

where

$V$  = voltage applied to the heater (reference balance vessel)

$R$  = resistance of the electrical heater

The absolute sensitivity of the adiabatic calorimeter has been reported to be  $2.1 \text{ J h}^{-1}$  and this device is usually designed to handle a small liquid volume ( $250 \text{ cm}^3$ )<sup>46</sup>. This is a comparatively elaborate device and the calculation of the integral in Eq. (19) is a somewhat bothersome task. It is also impractical to withdraw samples from the reaction vessel during the course of an experiment. As a result, parallel incubation is necessary if sampling is to be carried out. The application of adiabatic calorimeters is limited to experiments in batch culture.

#### 4.1.2 Thermal Fluxmeter

Unlike adiabatic calorimeters where the reaction vessel is isolated, a thermal fluxmeter allows heat to flow along a controlled path to a heat sink. Heat flow rate is detected by monitoring the temperature gradient along this path. The measuring element in such calorimeters is a multiple-junction thermocouple (up to 10,000 electrolytically formed junctions have been employed) and the thermopile is also the controlled path through which heat passes. It is worthy of mention that when the junctions of the thermopile are maintained at different temperatures, an e.m.f. (electromotive force) is produced. Conversely, when a current is applied to a thermopile one set of junctions becomes hotter and the other set cooler. The direction and the magnitude of the temperature gradient depends on the polarity and the magnitude of the impressed current (the Peltier effect).

Similar to the adiabatic calorimeter, the twin calorimeter is employed and the measuring element of the reaction vessel is compared with that of an identical vessel. The design and operation of the thermal fluxmeter has been extensively described by its developers<sup>47,48,49,50</sup>.

The absolute sensitivity of the thermal fluxmeter, which is designed to handle a very small sample ( $10 \text{ cm}^3$ ), is higher than that of the adiabatic calorimeter. About  $0.042 \text{ J h}^{-1}$  is very easily detectable<sup>46</sup>. However, there is some zero drift at this

sensitivity level. Since the thermal fluxmeter is designed for a general application some modifications to the standard design have been found necessary for microbial studies<sup>46)</sup>. Even though this type of instrument has been widely employed in microbial work it is a relatively difficult electrical measurement to make. Its application is also limited to experiments in batch culture.

#### 4.1.3 Flow Calorimeter

Recently, Eriksson and Wadsö<sup>51)</sup> and Eriksson and Holme<sup>7)</sup> described a flow calorimeter which can be used to measure heat evolution, independent of the disturbances caused by stirring or the addition of gas, alkali or nutrients. The calorimetric cell is coupled with an external reaction vessel of unrestricted volume via a pumped flow. The heat generated in the reaction vessel passes through thermopiles surrounding the measuring cell, generating an electrical potential, which, after amplification, is recorded as a heat flow rate.

Except for the wall growth, this design appears very applicable for anaerobic continuous culture system. For aerobic batch cultures, this technique suffers from the problem of oxygen deficiency. Such a drawback may be partly overcome by introduction of oxygen to the flow. As well, flow calorimeter may also be difficult to use with filamentous organisms or non-Newtonian fluids.

In summary, calorimetry is very useful to detect a small heat change since the technique is capable of high sensitivity. In the past, conventional calorimeters are limited to experiments in batch cultures. Since the development of flow calorimeters growth studies of continuous culture system are quite possible.

## 4.2 Dynamic Calorimetry\*

A successful attempt was reported by Cooney et al.<sup>1)</sup> based on a simple technique for measuring the rate of heat production during a microbial process by monitoring the broth temperature increase when the temperature controller was turned off. A sensitive thermistor was incorporated into one leg of a Wheatstone bridge to record the rise in temperature as function of time. The sensitivity of the circuit was such that full scale, on a 30 cm recorder, corresponded to 0.772 °C when the bioreactor was operated at 37 °C, and 0.690 °C at 30 °C.

$$Q_{acc} = \Sigma M_i C_{pi} \frac{dT}{dt} \quad (20)$$

where

$$\Sigma M_i C_{pi} = (MC_p)_{broth} + (MC_p)_{bioreactor\ jar} + (MC_p)_{stainless\ steel} \quad (21)$$

The heat accumulation measured in this manner was then corrected for heat losses and gains on the bioreactor.

$$Q_f = Q_{acc} - Q_{agi} + Q_{surr} + Q_{sens} + Q_{evp} \quad (22)$$



where

- $Q_f$  = heat evolution during the microbial process  
 $Q_{acc}$  = heat accumulation in the bioreactor with no temperature control  
 $Q_{surr}$  = heat loss to the surroundings  
 $Q_{sens}$  = heat gained by the gas stream leaving the bioreactor with respect to the heat content of the gas stream entering  
 $Q_{evp}$  = heat loss due to evaporation of water from the liquid culture  
 $Q_{agi}$  = heat of agitation (mechanical mixing power input)

If the incoming gaseous stream is saturated with water at the temperature of the culture broth,  $Q_{sens}$  and  $Q_{evp}$  are negligible. Equation (22), therefore, becomes:

$$Q_f = Q_{acc} - Q_{agi} + Q_{surr} \quad (23)$$

Since the impeller rotational speed and aeration rate is kept constant during the culture growth,  $(Q_{agi} - Q_{surr})$  is calibrated before inoculation at the specified agitation and aeration rates

$$Q_f = Q_{acc} - Q_{agi} + Q_{surr} = 0 \quad (24)$$

Hence

$$Q_{agi} - Q_{surr} = (Q_{acc})_{t < 0} \quad (25)$$

Therefore, the metabolic heat at time  $t$  is determined by

$$(Q_f)_t = (Q_{acc})_t - (Q_{acc})_{t < 0} \quad (26)$$

The overall accuracy of the dynamic calorimetric technique is reported to be  $-1.2\%$ . When the quantity of heat release is around  $20 \text{ MJ m}^{-3} \text{ h}^{-1}$  the accuracy of dynamic calorimetry ranges from  $-4.5\%$  to  $1.1\%$ .

The use of the dynamic calorimetric technique possesses a certain advantage in its relative simplicity. The approach, however, requires continuous attention during the experiment and may not be compatible with the application of automatic process control. This represents a serious disadvantage since microbial experiments or production runs can last for several days. The use of dynamic calorimetry represents a further experimental disadvantage. The temperature fluctuations resulting from the application of dynamic calorimetric technique may disturb the microbial activities. Furthermore, it usually takes a long time to obtain the heat evolution data by this approach. An initial period of 5–9 minutes is required to let the temperature rise reach a constant rate after the control system has been turned off. Dynamic calorimetry, therefore, is not as efficient in reliably determining the heat evolution during the end of the exponential culture growth phase when the rate of heat release may drastically decline in a relatively short period of time or during other rapid metabolic changes.