COMPUTERIZED ESTIMATION OF BACTERIAL HEAT PRODUCTION IN A LABORATORY FERMENTER

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A Dewar flask serves as a laboratory fermenter capable of supporting bacterial growth over the temperature range from ambient to greater than 70°C. These thermophilic temperatures are generated by the growing bacteria, and data from the temperature-time curves can be fitted into a computerized program developed for the apparatus to estimate the rate of bacterial heat generation and the cumulative heat generated by the microbial activity.

INTRODUCTION

Available methods for estimating the heat production on a dynamic basis in laboratory fermentations tend to be too expensive or too elaborate (Skinner 1969; Wadso 1974). Very few reports are available using simple techniques (Cooney et al. 1968).

A heat balance equation is used to describe microbial heat production in a laboratory fermenter. Such a description, utilizing small-scale, inexpensive laboratory equipment, is necessary to facilitate investigations of microorganisms responsible for the heat generated during composting (Poincelot 1972) and the thermophilic processing of liquid wastes (Popel and Ohnmacht 1972; Coulthard and Hendren 1973) as performed in large pilot plant fermenters.

Aerobic bacteria growing in the nutrient broth contained within the fermenter require both a constant air supply and agitation. The temperature of the fermenting broth varies with time according to the activity of the bacteria. By recording and analyzing this temperature-time curve, the dynamic heat production rate versus time and as cumulative heat production versus time.

MATERIALS AND METHODS

Growth Apparatus

A 4-L Dewar flask (Fisher Sci.) served as the fermenter. Heat loss was reduced by covering the top of the fermenter with a 2-cm thick plexiglas lid. An operating volume of 2 L was agitated vigorously with a 5000 rpm, 1/100 HP electric motor (Little Giant Pump Co.), driving a 3-bladed propeller of 4.5-cm diameter attached to a stainless steel shaft 35 cm long. Air was supplied by a Bell and Gosset Duraire pump (ITT, Monroe, La.) connected by tygon tubing to a flow meter (R. Gilmont Co.) and humidified by bubbling through water at room temperature. The humidified air was dispersed into the broth through a porous stone sparger located below the stirrer. A schematic diagram of the apparatus is presented in Fig. 1.

Organisms and Substrate

Experiments were conducted using Trypticase Soy Broth (BBL (30 g/L)) as the growth substrate. A mixture of organisms contained in thermophilically fermented dairy cattle manure was used as inoculum into the broth at ambient temperature (23°C).

Heat Measurement

Temperatures of both the stirred broth and the room air were simultaneously recorded using copper-constantan thermocouples and a Riken Denshi 10-inch (25.4-cm) strip chart recorder against the reference junction located in an ice bath. The temperature changes recorded in the broth were a function of the microbial activity. The shape of the temperature-time curve thus obtained depends on the following transient state equation.

\[ q = \frac{MCdT}{dT} + UA(t-T_\infty) + q_{\text{evap}} \]

where

- \( q \) = heat production rate of the bacteria
- \( q_{\text{evap}} \) = heat rate of evaporation of water
- \( q_{\text{sen}} \) = sensible heat rate of the bubbling air
- \( q_{\text{motor}} \) = heat rate of the stirrer
- \( T \) = temperature of the broth
- \( T_\infty \) = ambient temperature
- \( dT \) = slope of the temperature-time

Figure 1. Schematic diagram of fermentation apparatus for determining heat production by bacteria growing in liquid culture over mesophilic and thermophilic temperature ranges.
RESULTS

Two runs were performed to determine $q_{\text{motor}}$. The motor ran at constant speed with an 80-W electrical input. The average $q_{\text{motor}} = 2.75 \pm 0.25$ W. The average coefficient of convective heat loss ($UA$) value of the system was calculated from four runs. The average $UA = 0.30 \pm 0.05$ W/C.

The dynamic heat production of a bacterial growth experiment was calculated using Eq. 2, and the temperature-time curve, and the measured values of $q_{\text{motor}}$ and $UA$. Temperature and time were read from the strip chart record and entered on computer cards. The computer program used these data, the $q_{\text{motor}}$, $UA$ and $MC$ previously calculated, and determined the heat production rate and the cumulative heat production due to the growth and metabolism of the bacteria.

The computer plots of temperature, cumulative heat production and rate of heat production by bacteria growing in liquid culture are shown in Fig. 2.

DISCUSSION

A temperature exceeding 60°C was produced in the laboratory fermenter within 24 h (Fig. 2). Temperatures have exceeded 70°C in other experiments (unpublished observations). Similar rises in temperature within 48 h have been reported for solids composting of moistened straw (Norman et al. 1941; Carlyle and Norman 1941), and of refuse (Niese 1963) using Dewar flasks as the fermentation vessels. These and similar studies (Rothenback 1961) on the heat output of thermophilic bacteria growing on wool have used adiabatic apparatus consisting of a culture vessel containing within an incubator. The latter requires precise electronic monitoring and control to maintain its temperature precisely with that of the culture vessel.

There is little information concerning the production of high temperatures by bacteria growing in liquid culture. Most studies on the heat output utilize an expensive microcalorimeter (Skinner

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The cumulative heat production produced by the bacteria growing over the wide temperature range discussed above is also shown in Fig. 2. Approximately 420 kJ of heat were produced within the first 16 h of incubation. The cumulative heat production exceeded 544 kJ by 24 h. This figure represents a typical fermentation pattern corresponding to the so-called exponential growth equation (Aiba et al. 1965).

It is evident that the maximum rate of heat production by the growing bacteria was achieved during the period 10-12 h after the start of the experiment (Fig. 2). This maximum rate of approximately 40.6 kJ/h (5.65 X 10^-3 W/mL) was reached when the temperature of the fermenter was in the range of 48°-54°C. The heating rate decreased after 12 h, and reached a minimum of 10.5 kJ/h (14.7 x 10^-4 W/mL) about 17-18 h after the start of the experiment. Although the heating rate of the bacteria decreased during this time period, the temperature of the fermenter rose to about 63°C. At 17.5 h, additional substrate (TSB) was added to the fermenter, resulting in an immediate increase in the heating rate of the bacteria and an increase in temperature to 65°C within the next 6.5 h. Measuring the heating rate is probably the easiest means of detecting the immediate effects of additions of substrates or inhibitors on the activity of the bacteria under study (Cooney et al. 1968; Walsh and Townsley, in prep.).

Although cell numbers were not determined for this particular experiment, similar experiments (Walsh and Townsley, in prep.) have indicated bacterial numbers in the range of 0.45-3.4 x 10^9 colony-forming units per mL (cfu/mL) at temperatures from 50° to 54°C. Assuming a bacterial count of 1.0 x 10^9 cfu/mL, calculations from the above data would indicate maximum heat outputs around 56.5 x 10^-12 W/cfu in the same temperature range. This value falls within the following ranges of bacterial heat outputs taken from the literature: 2.2-168 x 10^-12 W/cfu calculated from data on different species of bacteria growing in milk (Berridge et al. 1974). 6.7-3977 x 10^-12 W/cell for a mixture of organisms growing on wheat straw (Carlyle and Norman 1941).

Alternatively, knowledge of the heating rates can allow calculations to determine close approximations of the numbers of viable bacteria at given times or temperatures.

Although further refinement is needed for more precise work, the apparatus described does provide a practical means of studying the organisms involved in composting or in thermophilic fermentations. The computer evaluation of heating rates allows study on the effects of particular substrates, inhibitors and growth conditions (Walsh and Townsley, in prep.) on the achievement of thermophilic temperatures by microorganisms.

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